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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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Vol. 15, No. 1, February 2024

Editorial

Review Article

Original Articles

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Short Communication

Commentary

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Editorial

What are the strategies for national health security in preparation for the next pandemic?

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Reflecting on the past 4 years of the response to coronavirus disease 2019 (COVID-19), there has been considerable discussion about the importance of public health measures before vaccines and treatments become available during a new outbreak. Additionally, there has been a focus on transforming the patient-centered healthcare system to reduce mortality caused by the paralysis of medical services during a pandemic, as well as on protecting vulnerable populations affected by social inequality. It is widely recognized that improving governance is essential for these 3 strategies to be effectively implemented, and that ensuring human dignity as a fundamental human right is critical in the measures taken to respond to infectious diseases. However, medical interventions constitute the cornerstone of the response. The evolution of viruses has outpaced our predictions, reducing the effectiveness of treatments and vaccines by half or more. As a result, our response has been constrained to temporary and short-term solutions, constantly trying to catch up with the evolving threat. What, then, are the strategies for a more effective response in the face of the next outbreak? Discussions within the biomedical science community are particularly important, underscoring the need for concerted efforts to establish robust national health security governance.

Firstly, to reduce unexpected uncertainties, the "prototype pathogen approach" is crucial. This strategy involves preemptively predicting which diseases might reach epidemic levels, conducting preliminary research, and then swiftly developing a "medical countermeasure" strategy through further research as the situation evolves. This approach is in line with the World Health Organization's "A scientific framework for epidemic and pandemic research preparedness" consultation on the vaccine research response to pathogen X [1], as well as the United States National Institutes of Health's Workshop on Pandemic Preparedness: The Prototype Pathogen Approach to Accelerate Medical Countermeasures—Vaccines and Monoclonal Antibodies [2]. Both initiatives emphasize compiling a list of diseases with the potential to cause future pandemics, particularly as a strategy for vaccine and treatment development. For instance, directing research efforts toward prototype viruses within certain virus families that could spawn new pandemic pathogens (such as Coronaviridae, including severe acute respiratory syndrome coronavirus-1 [SARS-CoV-1], Middle East respiratory syndrome [MERS] coronavirus, and SARS-CoV-2; Orthomyxoviridae, including influenza; and Paramyxoviridae, including Nipah and respiratory syncytial virus) is a strategy that would necessitate the establishment of research centers focused on approximately 10 virus families currently under discussion.

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Secondly, this research center-focused approach requires a multidisciplinary effort as a complementary strategy to the "prototype pathogen approach," which can be broadly applied to various viruses within a classification group, including those that are yet to be discovered. Furthermore, the development of medical countermeasures (MCMs) based on prototype pathogens requires a robust collaborative network of scientists to advance promising candidates into clinical trials, drawing on foundational research in virus biology, pathogenesis, and immunity. This opens up opportunities for researchers from diverse disciplines or those with specialized technical skills to collaborate in innovative ways. Progress in developing MCMs for one prototype pathogen can be accelerated by leveraging insights and discoveries from research on other prototype pathogens.

Thirdly, research outcomes must be translated into MCMs that facilitate product development. This involves enabling technologies such as mRNA vaccines, recombinant virus vaccines, new adjuvants, or novel drug delivery systems. Additionally, innovations in vaccine storage and cold chain logistics necessitate the alignment of materials, components, and equipment to scale up production. Falling behind in foundational technology development could lead to a loss of market dominance, similar to the situation with semiconductors. Thus, there is a need for intensive investment in mRNA technology, which can be used for essential immunization vaccines, other therapeutic vaccines, and cancer treatments, because the market is expected to reach 230 trillion dollars by 2035 [3]. Our failure to produce significant results during this outbreak can largely be attributed to insufficient investment in foundational technologies, workforce development, university startups, and the cultivation of industry clusters related to materials, components, and equipment, which has left us trailing behind other nations.

In conclusion, the preparation for pandemics should not only focus on purpose-driven science in priority areas but also on fostering scientific and organizational capabilities to accelerate the development of products from MCM candidates, potentially putting us ahead of other countries in developing our response strategies. In essence, a robust and consistent management system—or governance—is essential for a swift medical response within the framework of "National Health Security." Establishing and steering a coherent health security governance agenda is vital. This includes: (1) monitoring diseases, including those imported from abroad; (2) strengthened surveillance and proactive case identification; (3) investigations in response to outbreaks; (4) clinical research and diagnostic laboratory tests; (5) evaluations of non-medical and medical interventions; (6) clinical trials of treatments and vaccines as part of an always-ready "research and development scenario." The WHO has incorporated lessons from its experiences with SARS-CoV-1 and MERS into its field response research strategies for the COVID-19 pandemic. Following the MERS outbreak in 2015, the Republic of Korea undertook a Joint External Evaluation (JEE) with the WHO and the Global Health Security Agenda (GHSA) to identify 19 areas of preparedness and response under the revised International Health Regulations (IHR 2005), thereby strengthening public health measures related to MCMs [4]. Although a multi-ministerial forum and a government-wide Research and Development Fund for Infectious Disease Research (GFID) were established, strategies directly connected to product development fell short. Despite commitments to develop an mRNA vaccine domestically, progress has been sluggish, and even with some production, these vaccines have not been fully integrated into clinical practice. Reflecting on the historical trajectory of vaccine development, including both triumphs and setbacks as seen with COVID-19 [5], it is imperative to identify bottlenecks preventing vaccines from reaching the field in order to better prepare for future outbreaks and national health security threats.

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.

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Review Article

Predictors of outcomes 3 to 12 months after traumatic brain injury: a systematic review and meta-analysis

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ABSTRACT

The exact factors predicting outcomes following traumatic brain injury (TBI) remain elusive. In this systematic review and meta-analysis, we examined factors influencing outcomes in adult patients with TBI, from 3 months to 1 year after injury. A search of four electronic databases—PubMed, Scopus, Web of Science, and ScienceDirect—yielded 29 studies for review and 16 for meta-analysis, in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. In patients with TBI of any severity, mean differences were observed in age (8.72 years; 95% confidence interval [CI], 4.77–12.66 years), lymphocyte count (−0.15×10^9/L; 95% CI, −0.18 to −0.11), glucose levels (1.20 mmol/L; 95% CI, 0.73–1.68), and hemoglobin levels (−0.91 g/dL; 95% CI, −1.49 to −0.33) between those with favorable and unfavorable outcomes. The prevalence rates of unfavorable outcomes were as follows: abnormal cisterns, 65.7%; intracranial pressure above 20 mmHg, 52.9%; midline shift of 5 mm or more, 63%; hypotension, 71%; hypoxia, 86.8%; blood transfusion, 70.3%; and mechanical ventilation, 90%. Several predictors were strongly associated with outcome. Specifically, age, lymphocyte count, glucose level, hemoglobin level, severity of TBI, pupillary reaction, and type of injury were identified as potential predictors of long-term outcomes.

Keywords: Brain injuries, traumatic; Meta-analysis; Patient outcome assessment; Systematic review

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Introduction

Traumatic brain injury (TBI) represents a major cause of global morbidity and mortality. This condition has a profound impact on affected individuals [1], with both direct and indirect social and economic repercussions stemming from the substantial costs associated with medical care and rehabilitation, in addition to functional impairments and loss of productivity [2]. Annually, an estimated 10 million individuals are impacted by TBI, with some cases

necessitating hospitalization or resulting in death [3].

TBI is often referred to as a "hidden epidemic" because the long-term disabilities it causes are largely invisible [4]. Individuals who experience severe brain injuries may face permanent impairments that affect their ability to learn, work, and care for their families [5]. These impairments can rob them of productive years and lead to prolonged periods of debilitation. Moreover, TBI imposes a substantial financial toll on individuals, their families, and society at large. With increased prevention efforts, many lives could be spared, and the burden of disability could be substantially reduced [6]. Indicators such as unresponsive pupils, cerebral mass lesions, and the need for neurosurgical interventions have been associated with higher mortality rates and worse functional outcomes at the time of hospital discharge among patients who present to the emergency department [7]. This article details the factors and outcomes associated with such injuries.

Depending on their functional outcomes after TBI, individuals may regain essential skills and return to everyday activities. Assessing the probability of adverse functional outcomes following TBI is critical, as it provides patients, families, and healthcare providers with valuable prognostic insights that can guide decisions regarding the suitability of therapeutic interventions. Accurate outcome prediction can shape prognosis, influence treatment choices, and impact policy [8].

Most studies have identified age as the primary predictor of outcomes following head injury, although age appears to be a stronger indicator of mortality and survival than of functional outcomes [9]. Unlike findings from animal research, the majority of human studies have not supported sex as a predictor of outcomes [9,10]. In contrast, the importance of the Glasgow coma scale (GCS) and pupillary response as prognostic factors cannot be overstated, as these have been consistently identified as significant predictors in the literature. Indeed, 100% of studies evaluating pupillary response and 94% of those assessing GCS have reported these measures to be statistically significant in outcome prediction [9].

Outcomes following TBI have been extensively studied, but the results have been inconsistent. Some research has been focused on the immediate and short-term effects of TBI, examining the acute phase of the injury, outcomes of hospitalization, and early rehabilitation progress. However, the present study sought to clarify the long-term outcomes, which have received less attention in the current body of research. To identify the most reliable predictors of TBI outcomes, a systematic review and meta-analysis are necessary. These methods can provide a comprehensive and critical assessment of the factors that influence recovery from TBI. Understanding these factors will improve our knowledge of the prognosis for TBI and aid in the development of customized interventions to improve outcomes.

HIGHLIGHTS

- This study demonstrated that age, lymphocyte count, glucose level, hemoglobin level, Glasgow coma scale score, pupillary reaction, and type of injury can aid in predicting outcomes following traumatic brain injury.
- Variables such as neutrophil count, white blood cell count, and neutrophil-to-lymphocyte ratio were found to lack significance for outcome prediction.
- Predictors of outcomes 3 to 12 months after injury were examined.

The selection of a 3- to 12-month timeframe for the study was informed by the characteristics of TBIs and the research objectives. TBIs can lead to a diverse spectrum of outcomes that vary according to injury severity and the response of the patient to treatment and rehabilitation. The chosen period enabled the evaluation of extended recovery patterns. The 3-month minimum delay ensured that the study concentrated on individuals who had moved beyond the acute phase and were comparatively likely to have attained a plateau in their recuperation.

This review was conducted to identify significant factors influencing the outcomes of TBI and to ascertain which variables can predict patient outcomes within 3 to 12 months following TBI. By synthesizing data from numerous studies and identifying the most reliable predictors of TBI outcomes, this systematic review augments the existing body of knowledge on TBI prognosis and may improve patient care during the crucial recovery period of 3 to 12 months. Additionally, the review underscores the methodological weaknesses and constraints of the studies examined and suggests directions for future research.

Materials and Methods

This systematic review and meta-analysis was conducted on August 18, 2021, utilizing the methodology outlined in "A Systematic Review to Support Evidence-Based Medicine" [10]. The protocol was prospectively registered with the ResearchRegistry (Review Registry UIN: reviewregistry1500). In addition, we adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for the execution and documentation of meta-analyses [11]. The PRISMA statement includes a 27-item checklist and a

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4-phase flow diagram that includes identification, screening, eligibility, and inclusion. A critical component of this methodology is the development of a screening protocol with robust eligibility criteria to identify the most relevant studies pertaining to the research topic [12]. The research question for this study was designed to address the following primary concerns, based on population, exposure, and outcome: "Which studies have examined factors that predict outcomes 3 months after a traumatic brain injury?" and "What are these factors, and how significant are they in predicting outcomes?"

Eligibility Criteria

Studies of sufficient quality were concentrated on identifying predictors of outcomes in patients with TBI. These included observational and cross-sectional studies, as well as both prospective and retrospective research. Excluded from the analysis were conference presentations, case reports, interventions, and proceedings. Articles that failed to satisfy the inclusion criteria (Table 1) were also omitted. Studies were selected for inclusion if their titles contained the strategic keywords and if the title, abstract, and keywords were free of terms like "dark ages," "mice," "rat," or "ancient". The rationale for excluding these terms was to filter out studies that were not relevant to the current state of knowledge on TBIs. For example, studies that used historical data or animal models, or that focused on ancient treatments or practices. These terms were chosen based on a preliminary search of the literature, which revealed that they were often associated with such studies.

Information Sources

To comprehensively identify publications reporting on outcome predictors in patients with TBI, data were systematically gathered from major databases including PubMed, Scopus, Web of Science, and ScienceDirect. This effort was conducted in collaboration with search specialists and researchers within the field of TBI.

Search Strategy

The search process included only observational studies, excluding other article types. A total of 103 studies were identified via Science Direct, 247 through Scopus, 264 from Web of Science, and 170 through PubMed. This was achieved by searching study titles for the following keywords to ensure relevance to the research strategy: head trauma, head injury, brain trauma, TBI, traumatic head injury, predictors, predicting outcome, predict outcome, predictive factors, prediction factors, outcome prediction, survival prediction, and outcome predictors. Given the extensive number of studies, this title-based keyword search was necessary to meet the eligibility criteria of the research. The search was conducted from January 2017 to August 2021.

Selection Process and Data Collection

In the initial phase of article selection, 2 reviewers (Y.I. and M.C.) excluded articles with irrelevant titles or abstracts. Subsequently, the second phase involved a detailed review of the full texts of the articles to determine their eligibility based on the inclusion criteria. The organization and evaluation of titles and abstracts, as well as the identification of duplicate articles, were facilitated using Mendeley reference management software. Any disagreements that arose during each phase were settled through consensus with a third reviewer (E.S.). The full texts were independently assessed by 2 authors (K.B. and N.A.W.), who selected articles for inclusion based on mutual agreement that they satisfied the study criteria. Data extraction from the selected articles was performed by 2 reviewers (Y.I. and M.C.) using a standardized data collection form. For each study, details such as study characteristics, sample size, severity of TBI, and the scoring system used to measure outcomes were documented.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
All language papers published in peer-reviewed journals	Article reviews, book chapters, and case reports
Articles published between 2017 and 2021	Studies investigating nonhuman subjects
TBI severity of mild to severe	Studies including children
Studies investigating functional outcomes	Sample sizes of fewer than 20 participants
Outcomes categorized into favorable and unfavorable	Studies investigating outcomes other than favorable and/or unfavorable
	Studies including only a specific group of people or only a subgroup of TBI patients
	Articles that do not mention the site of the study
	Articles that do not mention the severity of TBI
	Outcomes at less than 3 months
	Penetrating TBI

TBI, traumatic brain injury.

Additionally, a meta-analysis was conducted of studies that investigated both favorable and unfavorable outcomes at 3 to 12 months following TBI.

Assessment of Study Risk of Bias

Two reviewers, N.E. and Y.M., assessed the quality of the articles using the Newcastle-Ottawa scale (NOS) [13]. The NOS awards a maximum of 9 points to studies with the lowest risk of bias across 3 domains: (1) the selection of study groups (which can earn up to 4 points); (2) the comparability of groups (worth up to 2 points); and (3) the ascertainment of exposure and outcomes (worth up to 3 points) [13], and this can be seen in Figure S1. Survey questions were formulated based on the NOS criteria, encompassing all 3 domains and enabling authors to provide details about their studies [14].

Synthesis Methods

A meta-analysis was conducted to estimate the differences between favorable and unfavorable outcomes in terms of predictive factors, utilizing rBiostatistics.com (Cloud Graphical User Interface for R Statistics and eLearning Platform, https://www.rbiostatistics.com, 2017) for the analysis of mean differences [15]. Both random and fixed-effects models were employed in the meta-analysis. Forest plots, illustrating outcomes after TBI along with 95% confidence intervals (CIs), were generated based on these models. Additionally, a meta-analysis of proportions was carried out using the Freeman-Tukey transformation [16] to compute the weighted summary proportion under both fixed and random effects models [17]. To investigate publication bias, statistical analysis was performed using Egger tests [18], which are methods employed in meta-analysis to detect the presence of publication bias.

Heterogeneity was assessed with the I-square (I^2) method [19,20]. I^2 represents the percentage of variability in results between studies due to heterogeneity rather than sampling error [21]. The magnitude of I^2 signifies the degree of heterogeneity: an I^2 value ranging from 50% to 75% suggests moderate heterogeneity, while an I^2 value exceeding 75% denotes high heterogeneity. Conversely, an I^2 value below 50% suggests homogeneity among the study results. Forest plots were employed to visually depict the extent of heterogeneity.

For 1-way analysis of variance (ANOVA), we utilized IBM SPSS ver. 25.0 (IBM Corp.) to determine whether any associations or differences existed between groups regarding the mean proportions of unfavorable outcomes. The Levene test [22] was employed to evaluate the homogeneity of variance among the studies. The purpose of this test was to ascertain whether the variances in the mean proportion of unfavorable outcomes differed significantly across the levels of TBI severity. According to the null hypothesis of the Levene test, the variances were equivalent across all studies. If the p-value obtained from the Levene test exceeded 0.05, this would suggest that variance was homogenous across the studies.

The least significant difference (LSD) test, also referred to as the Fisher LSD test, functioned as a *post hoc* analysis employed following ANOVA to ascertain which group means exhibited significant differences [23]. Upon achieving a significant outcome in ANOVA, the LSD test facilitated the pairwise comparison of mean values across TBI severity groups to identify those that differed significantly from each another.

Results

Study Selection

Figure 1 presents a modified PRISMA diagram, outlining the research process for study identification, selection, eligibility determination, and inclusion. This selection process yielded 784 articles, of which 307 were excluded due to duplication. The application of inclusion and exclusion criteria during the eligibility assessment of titles and abstracts narrowed the field to 127 papers. Subsequent full-text article reviews permitted the inclusion of 58 articles in the review, following the exclusion of an additional 69 articles.

Study Characteristics

Table 2 summarizes the characteristics of the included studies. Within the meta-analysis, 10 studies focused on the outcomes of individuals with severe TBI [24–33], 1 study addressed outcomes in those with mild TBI [34], 7 studies considered participants with moderate to severe TBI [35–41], and 11 studies encompassed all levels of TBI severity [42–52], assessing outcomes (including mortality) as either favorable or unfavorable. A variety of scales were employed to measure outcomes, such as the Glasgow outcome scale in 19 studies, the Glasgow outcome scale-extended in 9 studies, the quality of life after traumatic brain injury-overall scale in 1 study, the disability rating scale in 1 study, absolute functional gain in 1 study, the functional independence measure in 1 study, and the Rancho Los Amigos scale in 1 study, among others. Additional characteristics are detailed in Figure 1.

Risk of Bias in Studies

In nearly all cases, evaluating publication bias was not feasible due to the meta-analysis for each factor incorporating fewer than 8 studies. However, an assessment of publication bias was conducted specifically regarding sex. The findings from

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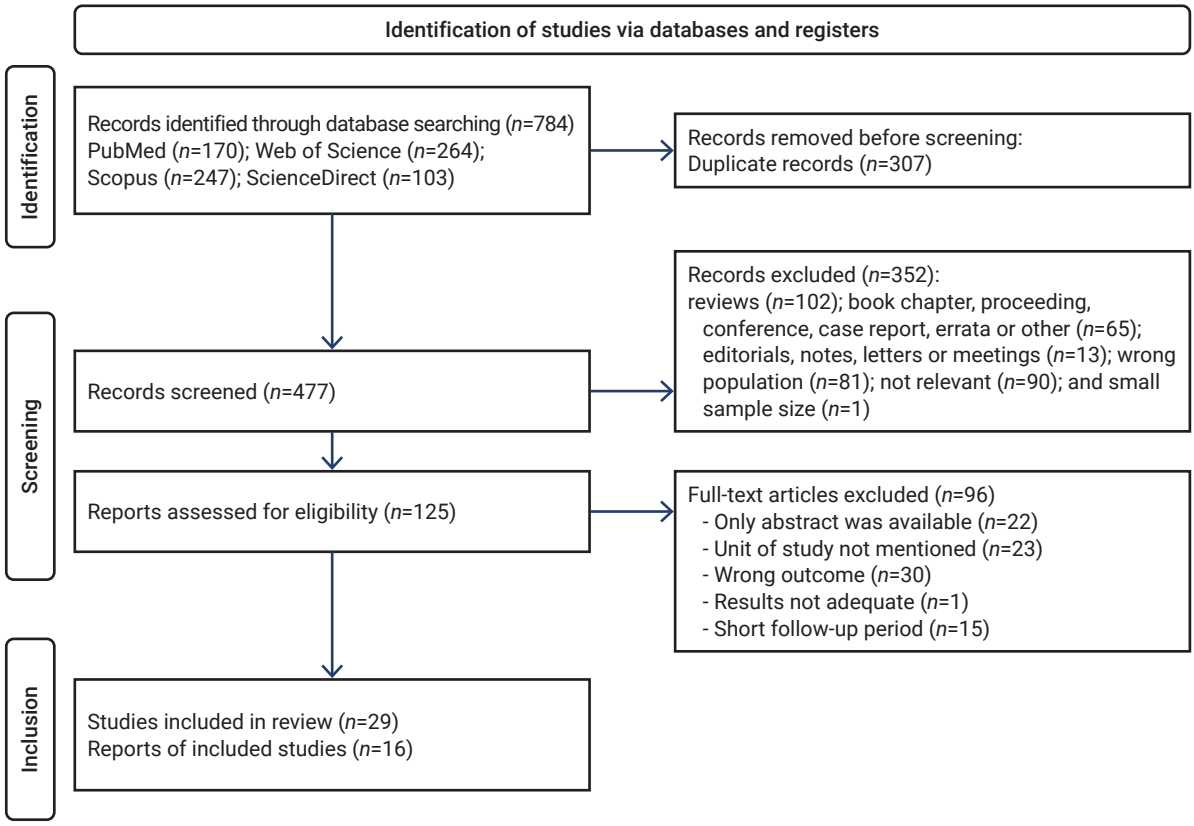

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram for predictors associated with traumatic brain injury. The PRISMA chart was designed to quantify these predictive factors.

the Egger test, which yielded a *p*-value of 0.775, suggest an absence of publication bias.

Results of Syntheses

The present study incorporated various factors, with an independent meta-analysis conducted for factors that were investigated in multiple studies. Forest plots for outcome predictors, as shown in Figure 2, revealed a significant mean difference in age of 8.72 years (95% CI, 4.77–12.66 years) between those with favorable and unfavorable outcomes. Additionally, the plots indicated significant mean differences for lymphocyte count (−0.15×10^9/L; 95% CI, −0.18 to −0.11), glucose levels (1.20 mmol/L; 95% CI, 0.73–1.68), and hemoglobin levels (−0.91 g/dL; 95% CI, −1.49 to −0.33). White blood cells (WBCs), however, did not exhibit a significant mean difference, with a pooled result of 1.74×10^9/L (95% CI, −0.76 to 4.24) across 4 studies. Neutrophils, analyzed in 2 articles, also showed no significant mean difference, with a result of 0.19×10^9/L (95% CI, −3.64 to 4.03). The neutr ophil-to-lymphocyte ratio (NLR) similarly did not demonstrate a significant mean difference, with a pooled result of 6.96 (95% CI, −3.54–17.46) based on 3 studies. Despite its frequent mention in the literature, GCS did not achieve a significant mean difference, with a result of −2.75 (95% CI, −6.03 to 0.53). The studies included in the present meta-analysis exhibited considerable heterogeneity. Furthermore, while age exhibited a significant mean difference, it also displayed substantial interstudy heterogeneity. This is most evident in the forest plots for each variable, presented in Figure 2.

Another comparison was conducted through a meta-analysis of proportions to investigate all factors predicting an unfavorable outcome. The prevalence rates of such outcomes among patients with abnormal cisterns, intracranial pressure (ICP) exceeding 20 mmHg, a midline shift of 5 mm or greater, hypotension, hypoxia, the requirement for blood transfusion, and the need for mechanical ventilation were 65.7%, 52.9%, 63%, 71%, 86.8%, 70.3%, and 90%, respectively.

As shown in Table 3, analysis of the patients with unfavorable outcomes revealed no significant differences in sex, injury severity score (ISS), motor score, Marshall score, type of surgery, or injury mechanism relative to those with favorable results [25,27,28,30,31,33,35,37,38,40–42,44–46,49,50]. However, significant differences were noted in several areas. Regarding GCS score, a distinction was evident between GCS

Table 2. Characteristics of included studies

Study	Year	Principal predictive factors of outcome	Study design	Location	TBI degree	Outcome assessment tool	Follow-up period (mo)	Sample size	Quality score (NOS)
Kokkinou et al. [35]	2020	Age	Longitudinal	Cyprus	Moderate to severe TBI	GOS-E and QOLIBRI-OS	6	203	Good
Latronico et al. [36]	2020	Posttraumatic cerebral infarction	Prospective	Italy	Moderate and severe TBI	GOS	6	143	Good
Bennis et al. [24]	2020	CRASH model and combined model	Retrospective	Netherlands	Severe TBI	GOS	6	45	Fair
Vijapur et al. [37]	2021	Innate immunity, soluble molecules, chemokines	Prospective	USA	Moderate to severe TBI	GOS and DRS	6–12	221	Fair
Dullaert et al. [51]	2020	CRASH model	Retrospective	Belgium	All severities	GOS	6	417	Fair
Jiang et al. [25]	2020	ICP, caspase 3	Prospective	China	Severe TBI	GOS	6	45 P and 25 C	Good
Thelin et al. [47]	2019	Age, glucose level, CT scan results, UCH-L1, GFAP, S100B	Prospective	Sweden	All severities	GOS	12	172	Good
Charry et al. [48]	2019	CT scan results, IMPACT model, CRASH model	Retrospective	Colombia	All severities	GOS	6	309	Fair
Hellstrom et al. [34]	2017	Brain imaging data, clinical data	Prospective	Norway	Mild TBI	GOS-E, RPQ, PHQ-9	12	147	Good
Riemann et al. [52]	2020	PRx and 20-LPRx	Retrospective	Germany	All severities	GOS	6	855	Fair
Khaki et al. [43]	2021	IMPACT base model, CT scan results	Retrospective	Sweden	All severities	GOS	12	158	Fair
Thelin et al. [26]	2017	CT scan results	Prospective	Sweden and Finland	Severe TBI	GOS	12	1115	Good
Lindblad et al. [27]	2021	Q_A, cerebrospinal fluid, serum	Prospective	Sweden	Severe TBI	GOS	6–12	90 P and 15 C	Good
Chen et al. [28]	2018	Age, GCS, glucose, hypoxia, pupillary reactivity, neurological deterioration, seizure, SpO_2, CT scan results, mechanical ventilation, intracranial surgery, NLR	Retrospective	China	Severe TBI	GOS	12	688	Fair
Petkus et al. [29]	2020	Age, duration of LCAI (PRx > 0.5), glucose, GCS	Retrospective	Lithuania	Severe TBI	GOS	6	81	Fair
Bilgi et al. [38]	2021	Age, time from injury to surgery, GCS, pupillary reactivity, hemoglobin, glucose, TLC, INR, blood transfusion, decompressive craniectomy	Prospective	India	Moderate and severe isolated head injury	GOS-E	6	96	Good
Bendinelli et al. [30]	2017	GCS, injury severity score, days in ICU, CT scan results	Prospective	Australia	Severe TBI	GOS-E	6	50	Good
Tolonen et al. [31]	2018	None	Retrospective	Finland	Severe TBI	GOS	6 and 12	28	Good

(Continued to the next page)

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Table 2. Continued

Study	Year	Principal predictive factors of outcome	Study design	Location	TBI degree	Outcome assessment tool	Follow-up period (mo)	Sample size	Quality score (NOS)
Gritti et al. [39]	2019	Oxford Handicap Scale score, posttraumatic cerebral infarction, posttraumatic hydrocephalus, hygroma, length of stay, invasive ventilation	Retrospective	Italy	Moderate and severe	GOS-E	12	193	Fair
Dolmans et al. [32]	2020	Age, hematocrit, hemoglobin, red blood cells, sodium, blood urea nitrogen, INR, prothrombin time	Retrospective	USA	Severe TBI	GOS	3	255	Fair
Bonow et al. [33]	2018	Age, race, education, mechanism of injury, motor score, pupillary reactivity, Abbreviated Injury Scale Head score, CT scan results, initial cerebral perfusion pressure	Prospective	Bolivia and Ecuador	Severe TBI	GOS-E	6	550	Good
Li et al. [49]	2018	Monocyte level	Observational and non-interventional	China	All severities	GOS-E	6	141	Good
Lindblad et al. [42]	2018	Age, GCS, pupil responsiveness, CT scan results, SpO_2, arachidonic acid receptor, thrombin receptor, activated partial thromboplastin time, platelet transfusion, COX inhibitor treatment	Retrospective observational	Sweden	All severities	GOS	12	178	Fair
Palekar et al. [44]	2021	CT scan results, GCS, pupillary reactivity	Prospective	India	All severities	GOS	1, 3, and 6	108	Good
Yao et al. [40]	2017	Age, GCS, motor score, pupillary reactivity, mass lesions, mass lesion size, intraventricular hemorrhage, suprasellar cisterns	Retrospective cohort	China	Moderate, severe, and complicated mild	GOS	3–6	302	Fair
Lagerstedt et al. [45]	2020	NA	Prospective, observational	Finland	All severities	GOS-E	6–12	88	Good

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Table 2. Continued

Study	Year	Principal predictive factors of outcome	Study design	Location	TBI degree	Outcome assessment tool	Follow-up period (mo)	Sample size	Quality score (NOS)
Zhao et al. [50]	2019	Age, mechanism of injury, pupillary reactivity, GCS, CT scan results, coagulopathy, WBC count, neutrophil ratio, lymphocyte ratio, NLR	Retrospective, observational	China	All severities	GOS	6	1291	Fair
Svedung Wettervik et al. [46]	2019	Age, GCS, pupillary reactivity, CT scan results, arterial blood pressure	Retrospective	Sweden	All severities	GOS-E	6	362	Fair
Al-Hassani et al. [41]	2018	Age	Retrospective observational	Qatar	Moderate to severe TBI	RLA, FIM, AFG, disability scale	3	201	Fair

TBI, traumatic brain injury; NOS, Newcastle-Ottawa scale; GOS-E, Glasgow outcome scale-extended; QOLIBRI-OS, quality of life after traumatic brain injury-overall scale; GOS, Glasgow outcome scale; DRS, disability rating scale; CRASH, Corticosteroid Randomization after Significant Head Injury; ICP, intracranial pressure; P, patients with TBI; C, controls; CT, computed tomography; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; PRx, pressure reactivity index; LPRx, long pressure reactivity index; IMPACT, International Mission for Prognosis and Clinical Trial; RPQ, Rivermead Post-Concussion Symptoms Questionnaire; PHQ-9, Patient Health Questionnaire-9; Q_A, CSF-serum albumin quotient; GCS, Glasgow coma scale; SpO_2, blood oxygen saturation; NLR, neutrophil-to-lymphocyte ratio; LCAI, longest cerebrovascular autoregulation impairment; TLC, total leukocyte count; INR, international normalized ratio; ICU, intensive care unit; COX, cyclooxygenase; NA, not applicable; WBC, white blood cell; RLA, Rancho Los Amigos; FIM, functional independence measure; AFG, absolute functional gain.

13–15, with a prevalence of unfavorable outcomes of 6.9% (95% CI, 2.535%–13.161%), and GCS 3–8, with a proportion of 58.6% (95% CI, 32.838%–82.035%). Pupillary reactions also differed significantly, with 31.8% (95% CI, 18.228%–47.153%) of patients with both pupils reactive demonstrating poor outcomes compared to 71.6% (95% CI, 53.901%–86.469%) among those with no pupillary reaction and 59.5% (95% CI, 51.214%–67.428%) of those with only 1 reactive pupil. Additionally, the type of injury was a significant factor, with 76.6% of those with intraventricular hemorrhage (IVH) experiencing poor outcomes (95% CI, 59.685%–89.971%) compared to 35.9% (95% CI, 19.776%–53.833%) of patients with intracerebral hematoma. When comparing epidural hematoma (24.9%; 95% CI, 18.079%–32.446%) and subdural hematoma (44.0%; 95% CI, 37.108%–51.097%) to IVH, significant differences were again observed (Table 3).

We explored the association between the severity of TBI and the rate of unfavorable outcomes from 3 months to 1 year after injury across all included studies. First, we confirmed the homogeneity of variances using the Levene test, which yielded a significance value of 0.628. Subsequently, we observed variations in the mean proportions of unfavorable outcomes across 3 categories of TBI severity: TBI of any severity, moderate to severe TBI, and severe TBI. To determine whether these differences were statistically significant, we employed 1-way ANOVA. The results revealed a significant disparity ($p = 0.032$) only between patients with TBI of any severity and those with severe TBI, as determined by the *post hoc* LSD test.

Discussion

Across the included studies, a significant association was found between the proportion of unfavorable outcomes and the GCS severity when investigating the link between poor outcomes and TBI severity. The studies that included individuals with severe TBI reported a significant proportion of patients experiencing unfavorable outcomes, aligning with findings from prior research. Patients with mild GCS scores exhibited the greatest frequency of favorable outcomes, whereas those with severe GCS scores experienced the highest mortality rates and the lowest rates of favorable outcomes [53].

The pooled results of this study indicate a disparity between the favorable and unfavorable groups, specifically suggesting that older patients with TBI are particularly likely to experience unfavorable outcomes. Age has been identified as a predictor of both mortality and unfavorable outcomes in several studies [29,33,35]. Separate research has substantiated the significant age difference between those with favorable and unfavorable outcomes [38,40],

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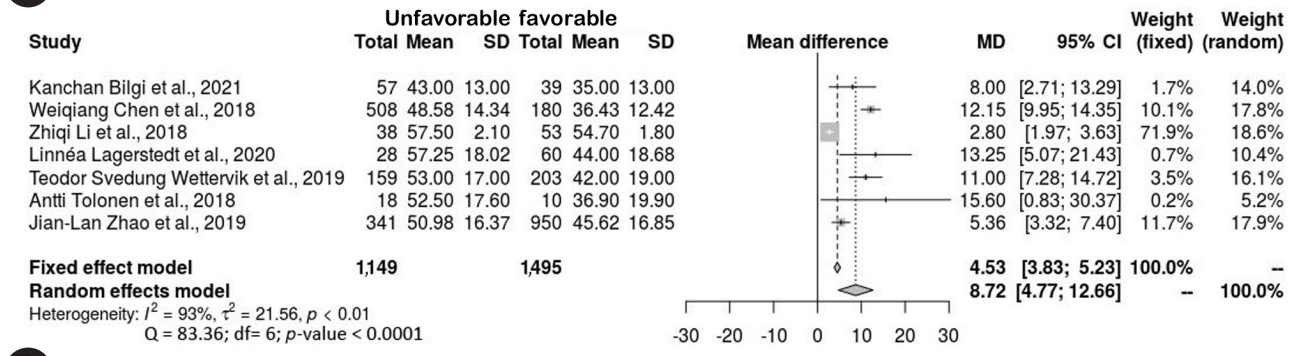
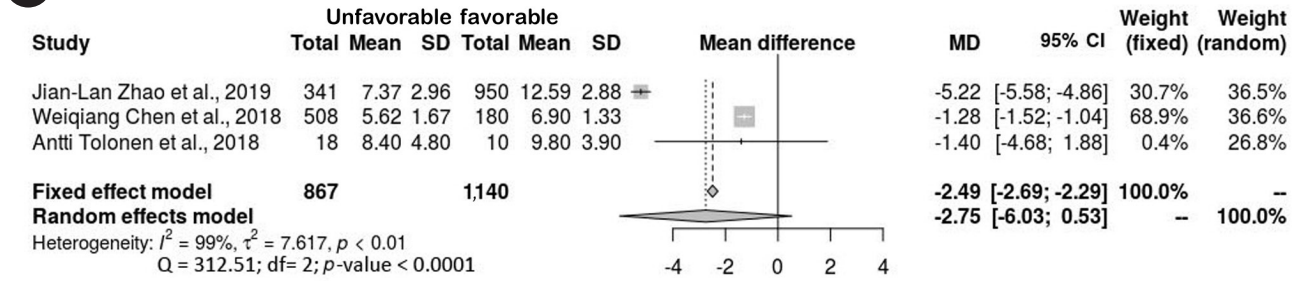
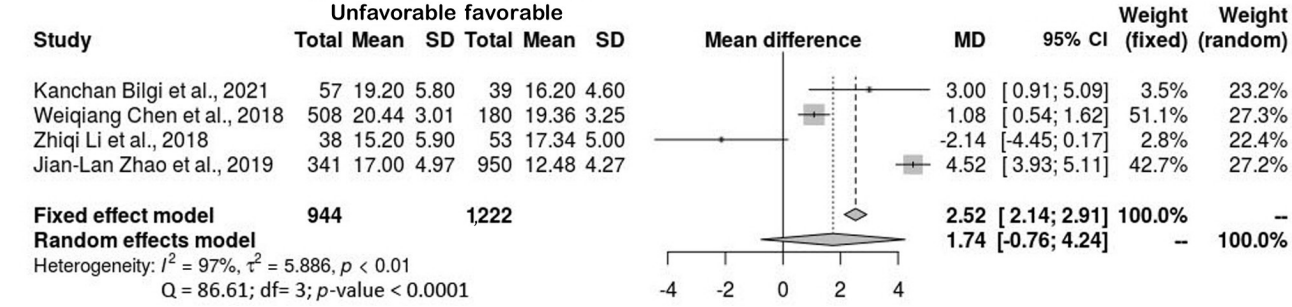
A Age and outcome



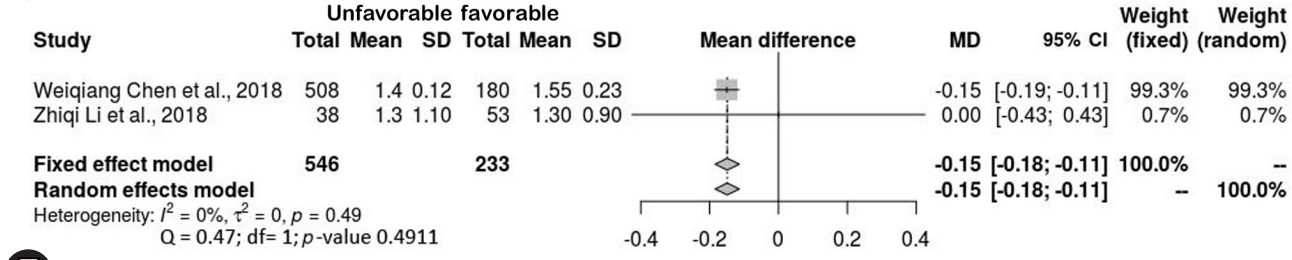
B Glasgow coma scale and outcome



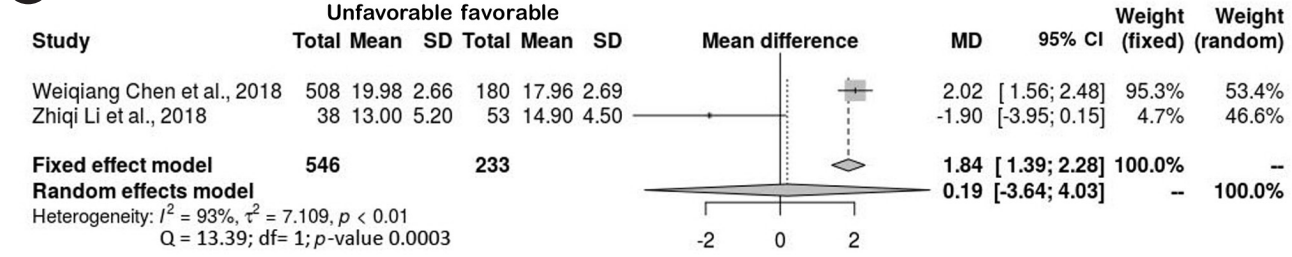
C White blood cells and outcome



D Lymphocyte and outcome



E Neutrophils and outcome



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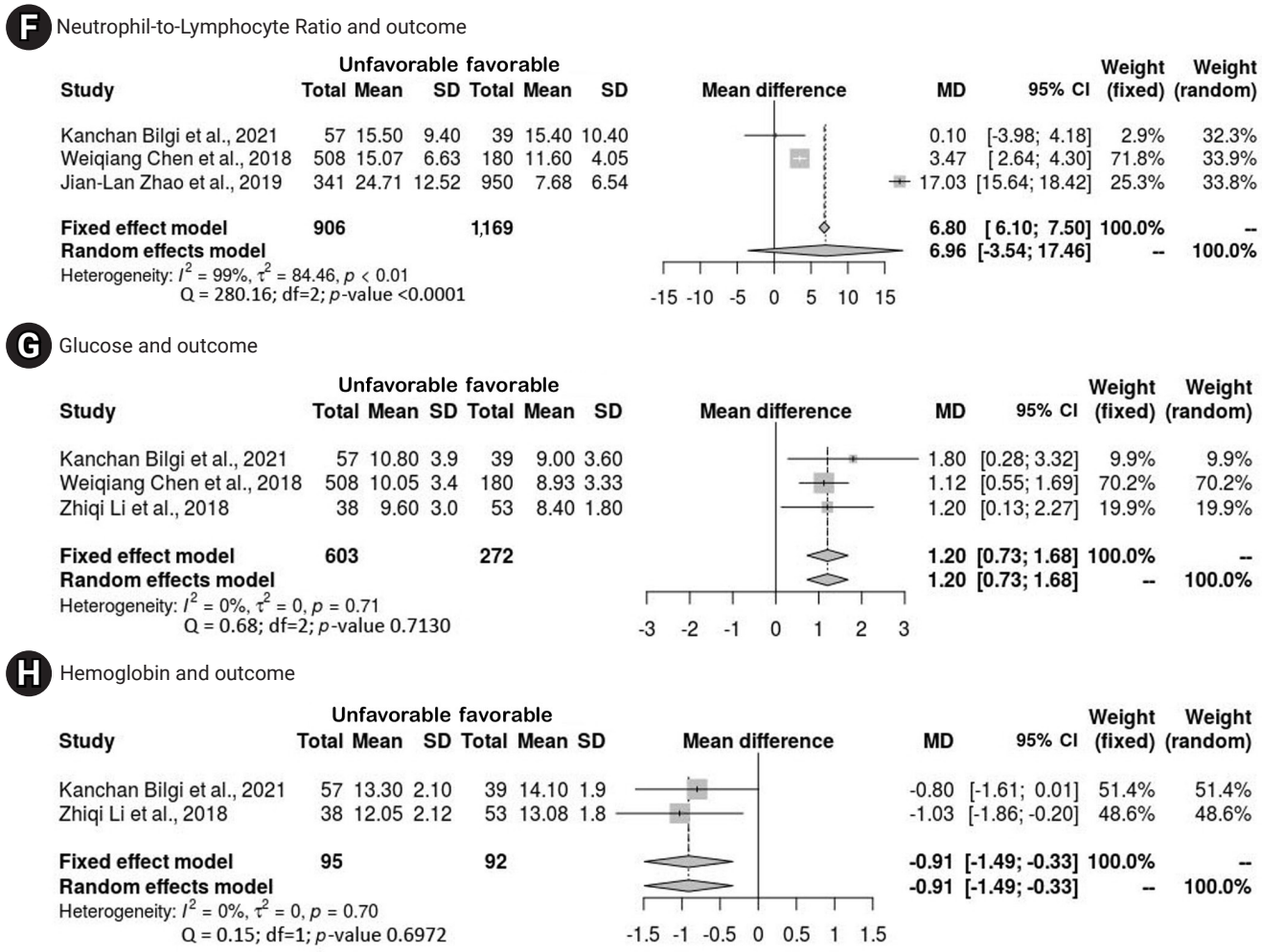

Figure 2. Forest plot of pooled mean differences (MDs), with 95% confidence intervals (CIs), of factors predicting outcomes 3 to 12 months after traumatic brain injury. (A) Age and outcome. (B) Glasgow coma scale and outcome. (C) White blood cell count and outcome. (D) Lymphocyte count and outcome. (E) Neutrophil count and outcome. (F) Neutrophil-to-lymphocyte ratio and outcome. (G) Glucose level and outcome. (H) Hemoglobin level and outcome. SD, standard deviation; df, degrees of freedom.

with age showing a significant correlation with outcomes at 6 months [40]. However, one study reported that the difference between individuals aged 21 to 40 years and those aged 41 to 60 years was not statistically significant [44]. In contrast, another investigation determined that age (odds ratio, 0.96; 95% CI, 0.93–0.98; p=0.005) independently predicted a positive functional outcome [41]. Overall, a substantial body of additional research [28,39,46,47,50] has indicated that age is a significant predictor of outcome.

Regarding the proportion of patients with unfavorable outcomes, no significant differences were observed in relation to ISS, sex, or mechanism of injury. ISS did not show a significant correlation with patient outcomes [42]. Similarly, sex did not exhibit any noticeable differences. While the frequency of injury severity did not significantly correlate with sex, a significant difference was evident in the occurrence of TBI between sexes [35]. No noticeable differences were found between favorable and unfavorable patient outcomes when considering sex [40]. The most common cause of injury was motor vehicle collision, and injuries most frequently occurred in public areas [35]. The mechanism of injury did not significantly relate to the proportion of patients with unfavorable outcomes. Although 71% of patients with hypotension experienced poor outcomes in our combined results, hypotension was not significantly associated with overall outcomes [33].

Between the groups with favorable and unfavorable results, significant differences were observed in median GCS score, GCS motor score, and pupillary reactivity [38,40]. GCS score upon admission, motor score, and pupillary reactivity have been identified as independent predictors of outcomes [46,47,50] or simply found to be associated

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Table 3. Proportions of unfavorable outcomes among patients with TBI according to various predictors of outcomes (unfavorable outcomes: random effects)

Variable	Studies (*n*)	Proportion (%)	95% CI	Test for heterogeneity	
				I^2 (%)	*p*
Sex [25,27,28,30,35,37,41,42,45,46,49]					
Male	11	47.098	34.423–59.966	97.62	<0.001
Female	11	55.355	39.858–70.336	94.58	<0.001
GCS [40,44,45]					
13–15	3	6.888	2.535–13.161	0.00	0.535
9–12	3	22.361	5.599–46.087	85.83	<0.001
3–8	3	58.584	32.838–82.035	91.91	<0.001
ISS [33,45]					
0 to 15	2	35.317	5.741–73.566	96.50	<0.001
16 or more	2	60.299	40.686–78.327	72.92	0.055
Motor score [33,40,46]					
1–2	3	57.074	14.514–93.932	98.25	<0.001
3–6	3	51.431	39.643–63.139	91.50	<0.001
Marshall score					
I or II [31,33,45,49]	4	34.433	14.880–57.252	84.67	0.002
III or IV [31,33,45,49]	4	54.197	39.698–68.343	65.48	0.034
V or VI [28,31,33,45,49]	5	62.488	50.174–74.033	82.52	<0.001
Abnormal cisterns [28,33,40]	3	65.713	48.943–80.675	96.45	<0.001
ICP >20 mmHg [33,49]	2	52.915	31.329–73.944	68.07	0.077
Midline shift of 5 mm or more [28,33,44]	3	63.063	48.815–76.235	87.92	<0.001
Hypotension present [33,49]	2	71.004	60.059–80.834	0.00	0.353
Hypoxia present [28,49]	2	86.785	77.704–93.767	0.00	0.406
Blood transfusion [38,49]	2	70.278	35.585–95.170	92.01	<0.001
Mechanical ventilation [28,49]	2	90.016	38.859–93.648	98.25	<0.001
Surgery					
Craniectomy [38,46,49]	3	62.456	43.356–79.732	74.71	0.019
Craniotomy [30,46,49]	3	50.511	32.933–68.023	73.51	0.023
Pupillary reaction					
Both reacting [33,40,44,49,50]	5	31.790	18.228–47.153	96.14	<0.001
Neither reacting [33,38,40,44,49,50]	6	71.647	53.901–86.469	87.95	<0.001
One reacting [33,38,40,44,49,50]	6	59.451	51.214–67.428	32.05	0.195
Mechanism					
Violence [31,49,50]	3	30.471	18.629–43.801	9.34	0.332
Road [31,33,49,50]	4	47.120	28.696–65.960	96.28	<0.001
Fall [31,33,49,50]	4	47.923	23.246–73.155	94.23	<0.001
Others [31,33,49,50]	4	28.353	9.265–52.835	83.35	<0.001
Type of injury					
DAI [30,44,50]	3	65.987	53.922–77.082	0.00	0.649
Epidural hematoma [40,49,50]	3	24.914	18.079–32.446	50.09	0.135
Skull fracture [33,50]	2	39.813	9.429–75.544	98.55	<0.001
Intracerebral hematoma [40,44]	2	35.885	19.776–53.833	55.92	0.132
Intraventricular hemorrhage [33,40]	2	76.586	59.685–89.971	47.48	0.168
Subarachnoid hemorrhage [28,33,44,49,50]	5	49.232	29.013–69.584	98.22	<0.001
Subdural hematoma [40,44,49,50]	4	44.043	37.108–51.097	53.49	0.092

TBI, traumatic brain injury; CI, confidence interval; GCS, Glasgow coma scale; ISS, injury severity score; ICP, intracranial pressure; DAI, diffuse axonal injury.

with outcomes [40,44]. Poor outcomes have been shown to be associated with low GCS scores at admission and low GCS motor scores [33,44]. In the present meta-analysis of proportions, a significant difference was found in the proportion of patients with unfavorable outcomes between those with both pupils reacting and those with 1 or no pupils reacting.

Within the subset of patients experiencing ICP exceeding 20 mmHg, 52.9% encountered unfavorable outcomes. A strong correlation was observed between elevated ICP and

both hospital mortality and long-term outcomes [33]. ICP was identified as a strong predictor of outcomes at 6 months following TBI [25]. Specifically, an average ICP above 21.3 mmHg throughout the monitoring period was associated with poor outcomes in certain patients [29]. Patients with poor outcomes exhibited significantly higher mean values of pressure reactivity index (PRx) and 20-minute-long pressure reactivity index (20-LPRx). While both PRx and 20-LPRx demonstrated statistical significance in predicting unfavorable outcomes through univariate regression analysis, only PRx maintained this significance in multivariate analysis [52]. Additionally, a mean PRx between 55 and 15, as well as a cerebral perfusion pressure (CPP) exceeding the optimal CPP threshold, were identified as significant independent predictors of outcomes [46]. The most critical prognostic indicators included GCS score, serum glucose levels, and the duration of the longest event of cerebrovascular autoregulation impairment, specifically when PRx exceeded 0.5 within 24 hours after admission [29].

The meta-analysis indicated that patients with poor outcomes exhibited significantly lower hemoglobin levels and lymphocyte ratios relative to those with favorable outcomes. Patients with favorable outcomes after TBI had significantly higher hemoglobin levels than those with poor results [49]. Furthermore, individuals with unfavorable outcomes presented with much lower lymphocyte ratios than their counterparts with favorable outcomes, although this finding was not specifically associated with outcomes at 6 months [50]. Significant differences were observed in blood transfusions, mean international normalized ratio (INR), and mean hemoglobin levels between the groups with favorable and unfavorable outcomes [38]. Patients with TBI who experienced poor outcomes also had markedly higher glucose levels. A significant difference was noted in the median mean glucose levels between the 2 outcome groups [38], with significantly lower levels in patients who had favorable outcomes [49]. Glucose levels were found to be substantially correlated with outcomes among the established International Mission for Prognosis and Clinical Trial predictors [47].

In the meta-analysis, no significant differences were observed in WBC counts, NLR, or neutrophil counts between the 2 outcome groups. Specifically, WBC counts were similar across both groups [49]. This finding contrasts with other research, where patients with poor outcomes exhibited significantly elevated WBC counts compared to those with favorable outcomes [50]. Similarly, although the results indicated no difference in neutrophil counts between groups [49], other studies have identified a significantly higher neutrophil ratio in patients with poor outcomes relative to those with favorable results [50]. The apparent discrepancies may be attributed to the substantial heterogeneity present within this meta-analysis.

Contrary to previous findings, subsequent research has shown that NLR at the time of admission remained a significant predictor of an unfavorable outcome 1 year later [28]. Furthermore, NLR was identified as an independent predictive factor for the 6-month prognosis of patients with TBI [50]. Although neutrophils, WBC count, and NLR did not demonstrate strong correlations with 6-month prognosis, patients with poor outcomes exhibited significantly higher NLRs compared to those with favorable outcomes [50]. We also found that the total leukocyte count (TLC) served as a predictor of both mortality and poor neurological outcomes at 6 months [38], with the mean TLC values differing substantially between the 2 groups [38]. Monocyte count at admission was linked to 6-month prognosis in patients with moderate to severe TBI, with higher monocyte counts observed in patients who experienced favorable outcomes [49]. Additionally, coagulopathy and caspase 3 were identified as highly significant predictors of outcomes 6 months after TBI [25,50].

Throughout this review, several methodological issues emerged that require attention. The diversity of predictor variables used in the research was particularly noteworthy. The initial aim of this study was to evaluate all known predictor variables for outcomes between 3 and 12 months; however, the existing research encompasses many predictors. For example, of the 29 studies included in the current analysis, 81 different predictor variables were identified. Another point of concern is the variation in methodological rigor. Building on insights from prior studies, the assessment of potential research for inclusion in this review revealed that the literature is characterized by numerous studies with small sample sizes, findings of statistical significance that lack clinical importance, and models derived from limited datasets. Furthermore, we noted frequent omissions in the reporting of sociodemographic information regarding study samples. This omission poses a serious challenge for researchers and clinicians who wish to generalize the findings to other populations.

A second issue was the variability in methodological quality. The existing literature is characterized by a multitude of analyses with small sample sizes, instances where statistical significance may have little clinical relevance, and models developed from small sample sizes, based on findings from previous research. Moreover, studies often did not report the sociodemographic characteristics of their samples. This omission similarly complicates the generalizability of the study's findings. Additionally, our review was limited by our

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method of retrieving reports, which relied on searching titles for keywords. We also found considerable heterogeneity among the studies included in the meta-analysis, which was most notably reflected in the varying degrees of TBI severity and the samples used in each study.

This study adhered to the PRISMA guidelines for the conduct and reporting of meta-analyses [11], and this can be seen in Supplementary Material 1 and 2 [11]. It was focused on identifying research published from January 2017 to August 2021 that examined factors predicting outcomes 3 months following TBI. Studies that met the eligibility criteria and demonstrated sufficient quality were selected, and their quality was evaluated using the NOS [13].

A meta-analysis of mean differences was utilized to estimate the disparity between favorable and unfavorable outcomes, as well as to produce forest plots employing both random-effects and fixed-effects models. Subsequently, a second meta-analysis was conducted, focusing on proportions. This analysis employed the Freeman-Tukey transformation [16] to compute the weighted summary proportion in both fixed and random effects models [17]. The potential for publication bias was statistically explored through the application of Egger tests [18], while heterogeneity was quantified using the I^2 method [19].

After confirming the homogeneity of variance across various studies with the Levene test [22], 1-way ANOVA was conducted to investigate any association between groups regarding the mean proportion of unfavorable outcomes. Subsequently, the LSD test was utilized to ascertain which group means differed significantly from each another [23].

Previous reviews have examined outcomes following TBI, with some focusing on long-term and others on short-term effects. Various aspects of recovery have been investigated, including functional outcomes—categorized as either favorable or unfavorable, and fatal or non-fatal—as well as return to work and social participation. These reviews identified factors such as NLR [54], level of education, age, mechanism of injury, psychiatric history [55,56], and injury severity [57] as good predictors of outcomes after TBI. In contrast, other studies found no differences in recovery rates between older and younger individuals [58] and reported no significant disparities in TBI mortality related to obesity [59]. The research concerning long-term outcomes after TBI is limited and varied [60], which may account for the inconsistency in identified predictors across reviews.

This meta-analysis examined predictors of long-term functional outcomes—favorable or unfavorable—following TBI of any severity at the time of hospital admission, incorporating both clinical and sociodemographic variables. The analysis revealed a set of predictive factors including age, lymphocyte count, glucose level, hemoglobin concentration, severity of TBI, pupillary response, and type of injury. Together, these factors are valuable in forecasting long-term outcomes that span from 3 to 12 months after injury. Within the scope of this review, significant heterogeneity was observed among several variables, which led to the exclusion of WBCs, neutrophils, neutrophil-lymphocyte ratio, and a range of other variables from consideration as potential predictors for long-term outcomes.

Conclusion

The findings of this study indicate that a range of factors are associated with unfavorable outcomes at 3 to 12 months, including advanced age, low GCS score upon admission, diminished lymphocyte count, elevated glucose level, increased NLR, the presence of mass lesions, IVH, high neutrophil ratio, elevated WBC count, high sodium concentration, elevated Oxford Handicap Scale score, increased monocyte count, and high hemoglobin level. These factors can assist healthcare professionals in estimating patient prognosis following TBI and enable them to predict outcomes swiftly and effectively, thereby facilitating timely and appropriate intervention. Although current evidence does not support the creation of a specific triage tool for predicting TBI severity based on each of these predictors, ongoing research may enable the development of such tools in the near future.

Supplementary Material

Supplementary Material 1. PRISMA 2020 abstract checklist. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses. **Supplementary Material 2.** PRISMA 2020 checklist. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses. **Figure S1.** Quality assessment of studies using a Newcastle-Ottawa scale for assessing studies in the systematic review and meta-analysis of the predictors of outcomes 3 to 12 months after traumatic brain injury. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0288.

Notes

Ethics Approval
Not applicable.

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

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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: YI, MC, ES; Data curation: YI, MG; Formal analysis: YI, MG; Investigation: YI, NAW, MA, NE, YM, KB, MG, AI; Methodology: YI; Project administration: YI, MC, ES; Resources: YI; Software: YI, MG; Supervision: MC, ES; Validation: ES, MC; Visualization: NE; Writing–original draft: YI; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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Original Article

Developing a national surveillance system for stroke and acute myocardial infarction using claims data in the Republic of Korea: a retrospective study

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Objectives: Limited information is available concerning the epidemiology of stroke and acute myocardial infarction (AMI) in the Republic of Korea. This study aimed to develop a national surveillance system to monitor the incidence of stroke and AMI using national claims data.
Methods: We developed and validated identification algorithms for stroke and AMI using claims data. This validation involved a 2-stage stratified sampling method with a review of medical records for sampled cases. The weighted positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the sampling structure and the corresponding sampling rates. Incident cases and the incidence rates of stroke and AMI in the Republic of Korea were estimated by applying the algorithms and weighted PPV and NPV to the 2018 National Health Insurance Service claims data.
Results: In total, 2,200 cases (1,086 stroke cases and 1,114 AMI cases) were sampled from the 2018 claims database. The sensitivity and specificity of the algorithms were 94.3% and 88.6%

for stroke and 97.9% and 90.1% for AMI, respectively. The estimated number of cases, including recurrent events, was 150,837 for stroke and 40,529 for AMI in 2018. The age- and sex-standardized incidence rate for stroke and AMI was 180.2 and 46.1 cases per 100,000 person-years, respectively, in 2018.

Conclusion: This study demonstrates the feasibility of developing a national surveillance system based on claims data and identification algorithms for stroke and AMI to monitor their incidence rates.

Keywords: Incidence; Myocardial infarction; Population characteristics; Population surveillance; Stroke

Introduction

An accurate computation of national health metrics, such as the incidence rates of stroke and acute myocardial infarction (AMI), is indispensable for crafting a robust national healthcare system adept at managing cardiovascular diseases [1–8]. In Korea, the National Health Insurance Service (NHIS) operates a comprehensive claims database that contains data from healthcare providers and insurers for reimbursement purposes. Given that this database covers the vast majority of the Korean population, it contains patient-level data pertaining to diagnoses, treatments, healthcare utilization, access, outcomes, and costs [9–13]. Consequently, this extensive dataset provides a promising foundation for establishing a national surveillance system for stroke and AMI.

Previous research efforts utilizing claims data to explore the incidence of stroke and AMI have encountered limitations in case identification. These challenges primarily originate from the use of International Classification of Diseases (ICD) codes, which are often not well-validated and are challenging to use for differentiating between acute and chronic events [14–22]. In 2004, there was an attempt to construct a surveillance system based on claims data, incorporating a validated diagnostic tool, to estimate the incidence of stroke and AMI. However, this system was predominantly centered around monitoring care quality at individual hospitals, relying heavily on ICD codes [23]. Since this attempt, there have been no additional endeavors to construct a similar system with national coverage. The present study seeks to fill this gap by constructing a national surveillance system for stroke and AMI, harnessing the NHIS claims data and employing validated identification algorithms for these conditions.

HIGHLIGHTS

- This study demonstrates the feasibility of creating a national surveillance system using claims data and identification algorithms to estimate the incidence of stroke and acute myocardial infarction.
- The age- and sex-standardized incidence rates stood at 180.2 per 100,000 person-years for stroke and 46.1 per 100,000 person-years for acute myocardial infarction.
- This system facilitates ongoing monitoring of the burden of stroke and cardiovascular disease in the Republic of Korea and aids in expanding nationwide epidemiological research.

Materials and Methods

Overview of the Study Process and the Organization of Study Teams

This study was launched with the aim of constructing a national surveillance system for the incidence of stroke and AMI as a private consignment project of the Korean Disease Control and Prevention Agency (KDCA). It required collaboration of a variety of organizations, encompassing (1) the Central Support Group of Cardiovascular Disease Management, Public Health and Medical Services, Seoul National University Hospital; (2) the Department of Biostatistics, Korea University College of Medicine; (3) the Korean Stroke Society, (4) the Korean Society for Preventive Medicine, and (5) the Korean Society of Cardiology. The structure of the study teams is illustrated in Figure 1A.

The study was conducted in the following steps: first, we formulated definitions for epidemiological indices pertaining to stroke and AMI. This was achieved through interactive consultations involving the Advisory Board for Disease Definition, 3 participating societies, and other experts (Figure 1A). Second, we designed identification algorithms for stroke and AMI based on claims data. Third, the developed algorithms underwent validation via a review of hospital records for cases selected from the NHIS claims data. Finally, we calculated the incidence of AMI and stroke in the Republic of Korea by applying the weighted positive predictive values (PPVs) and negative predictive values (NPVs) to the 2018 NHIS claims database (Figure 1B).

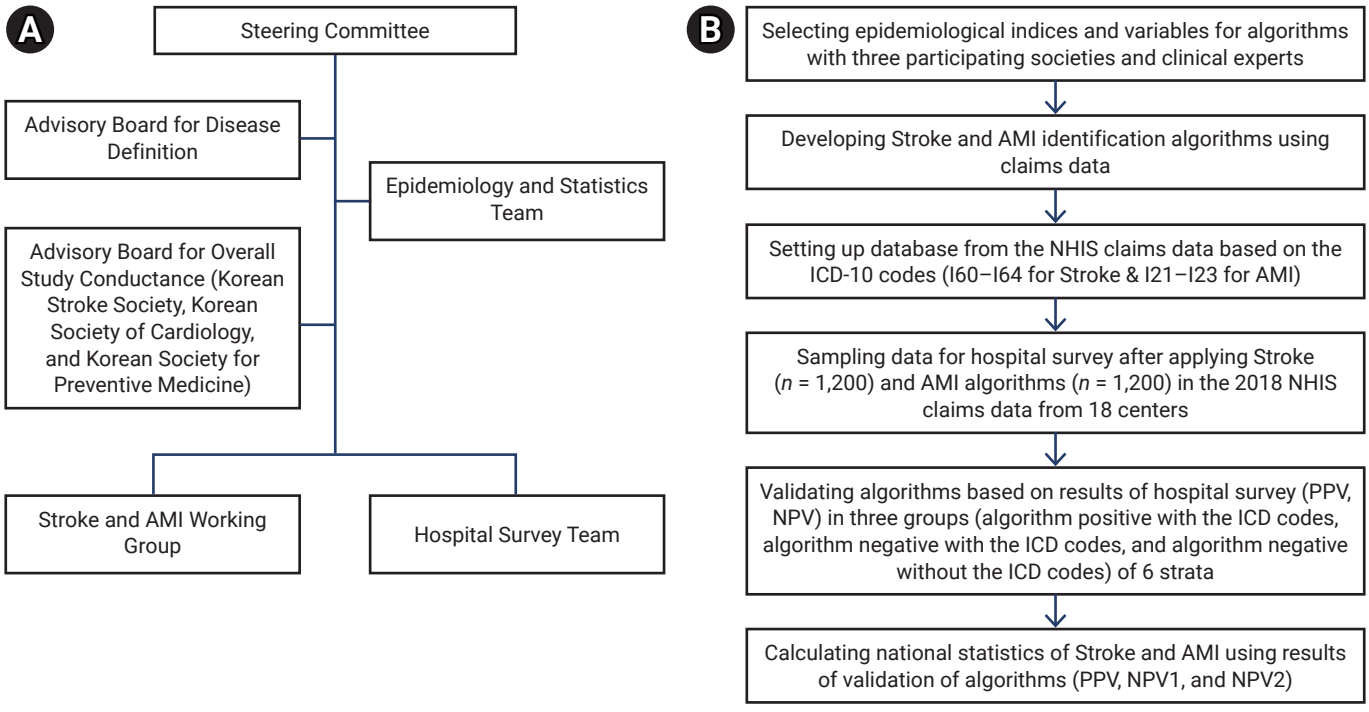

Figure 1. (A) Organization of the study teams. (B) Overview of the study process.
AMI, acute myocardial infarction; ICD, International Classification of Diseases; NHIS, National Health Insurance Service; PPV, positive predictive value; NPV, negative predictive value.

Epidemiological Indices, Definitions, and Identification Algorithms

To establish the epidemiological indices for stroke and AMI, we conducted an extensive review of the pertinent literature, including reports from the Organisation for Economic Co-operation and Development (OECD), the National Institute for Health and Care Excellence, and the OECD Health Care Quality Indicators project. Based on this review, we identified the incidence rate and proportion of stroke and AMI as the epidemiological indices for our study [24–27].

We defined stroke according to the World Health Organization (WHO) criteria, which delineate stroke as the rapid development of clinical signs indicating a focal or global disturbance of cerebral function lasting more than 24 hours or resulting in death, with no apparent cause other than of vascular origin [28].

Regarding AMI, we employed the fourth universal definition, which characterizes it as the presence of AMI accompanied by clinical evidence of acute myocardial ischemia and abnormal cardiac biomarkers [29].

The subsequent step was the development of identification algorithms. We selected relevant variables from claims data, based on our understanding of AMI and stroke management within the Korean healthcare setting. We designed an identification algorithm for stroke that encompassed both ischemic and hemorrhagic stroke cases, along with nontraumatic subdural hemorrhage. The algorithm was built around key identifiers, including ICD codes (I60–I64) for stroke and claims codes related to stroke diagnosis and treatments (Figure 2A). To apply this algorithm, we considered possible stroke admission episodes as a single claim or as combining claims if the gap between the discharge date of the first claim and the admission date of the second claim was 2 days or less. Claims with an interval of more than 2 days were deemed as separate episodes, irrespective of whether the consecutive claims were from the same hospital.

Similarly, we developed an identification algorithm for AMI. The algorithm's key identifiers included ICD diagnosis codes (I21–I23) for AMI and claims codes related to AMI diagnosis and treatments (Figure 2B). In applying this algorithm, possible AMI admission episodes were defined as a single claim or by combining claims if the gap between the discharge date of the first claim and the admission date of the second claim was 3 days or less, or if the gap in admission dates between the first and second claims did not exceed 28 days.

The epidemiological indices and identification algorithms with their key identifiers received approval from experts from the Korean Stroke Society, the Korean Society of Cardiology, and the Korean Society for Preventive Medicine (Figure 1B).

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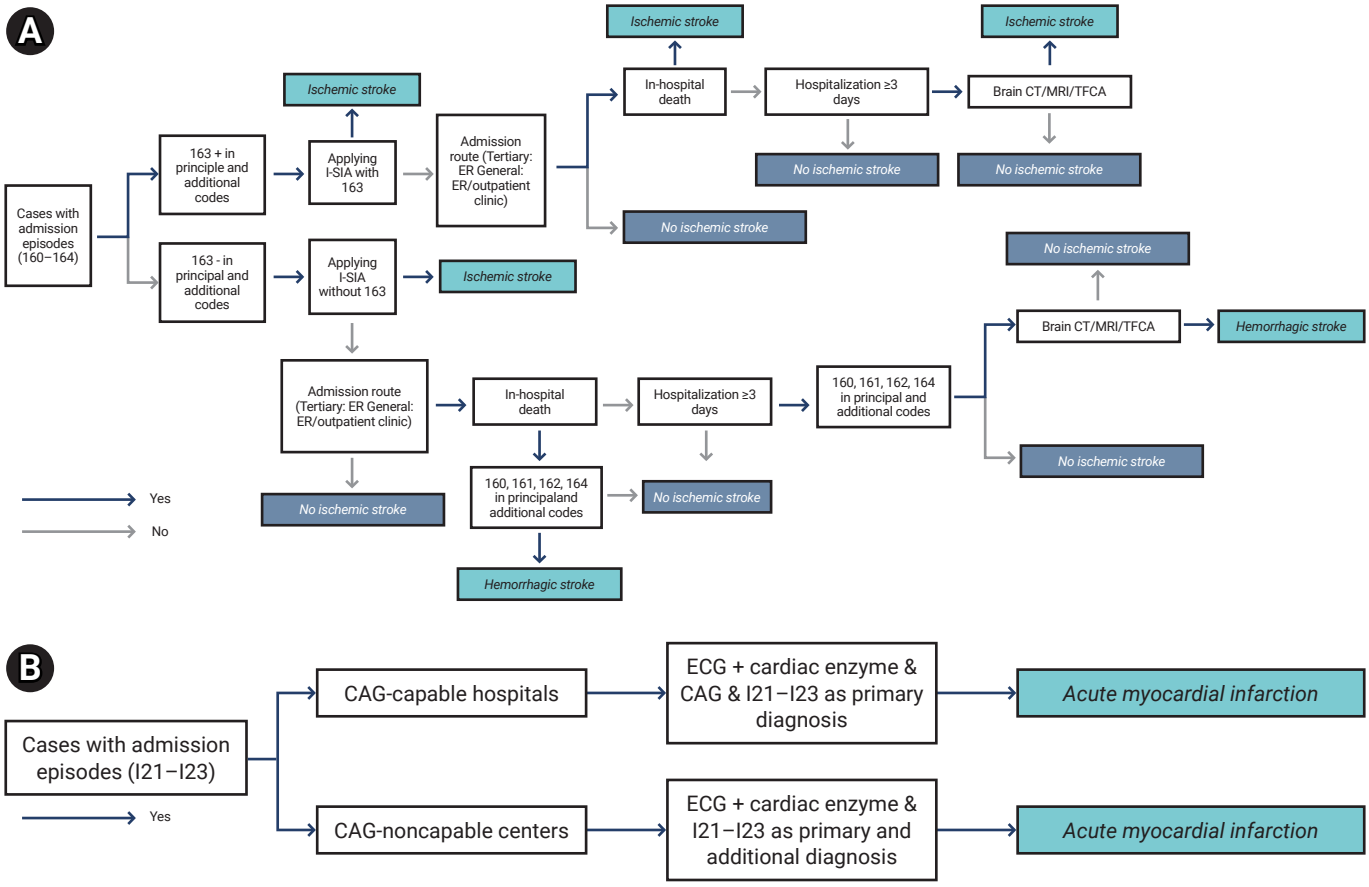

Figure 2. Identification algorithms for stroke and acute myocardial infarction (AMI).
(A) Any stroke identification algorithm (A-SIA). The stroke identification algorithm used in our study was a 2-stage process based on International Classification of Diseases (ICD) codes. In the first stage, the algorithm identified ischemic stroke cases, while the second stage focused on identifying hemorrhagic stroke cases. Each stage utilized a set of key identifiers associated with clinical practices during acute stroke management. A total of 23 key identifiers were employed in this algorithm. These identifiers included variables related to various aspects of stroke care, such as the admission route, stroke unit care procedures, brain imaging techniques (such as computed tomography [CT], CT angiography, brain magnetic resonance imaging [MRI], and transfemoral cerebral angiography [TFCA]) used for stroke diagnosis. It also included recanalization therapy (comprising intravenous thrombolysis and endovascular therapy) administered as hyperacute management post-ischemic stroke, antithrombotic therapy (including antiplatelet agents and anticoagulants), and interventional therapies (such as carotid endarterectomy or carotid and intracranial angioplasty/stenting) for secondary prevention after ischemic stroke. Surgical therapy, rehabilitation, and outcomes at discharge (such as length of stay and in-hospital mortality) were also incorporated into the key identifiers. I-SIA, ischemic stroke identification algorithm. (B) AMI identification algorithm. The AMI identification algorithm used in our study involved a classification process based on a hospital's capacity to perform coronary angiography (CAG). Subsequently, an AMI event was defined when the following conditions were met in CAG-capable hospitals: (1) diagnosis codes I21–I23 were present; (2) an electrocardiogram (ECG) was performed; (3) the serum troponin I or troponin T level was tested; and (4) CAG was performed during hospitalization. Conversely, in CAG-incapable hospitals, an AMI event was defined as follows: (1) diagnosis codes I21–I23 were present, (2) an ECG was performed, and (3) the patients underwent serum troponin I or troponin T testing during hospitalization.

Sampling

We applied 2-stage stratified sampling to select cases for the hospital survey [30,31]. Using the 2018 NHIS claims data, we defined admission episodes with and without the corresponding ICD codes for stroke (I60–I64) and AMI (I21–I23), as described earlier. These episodes were used for hospital and case selection.

In the first stage, we selected hospitals. Initially, we chose hospitals eligible for the survey from 6 administrative divisions—namely, Seoul, Gyeonggi, Daegu, Gyeongsangnam-do, Ulsan, and Busan—considering the feasibility of the survey. Subsequently, we determined the number of hospitals to invite for participation, considering the geographic regions and case availability. We also balanced the ratio of tertiary to general hospitals at 8:10 as guided by data from the 8th Acute Stroke Quality Assessment Program (ASQAP) [32]. The chosen hospitals were required to have over 20 episodes of both stroke and AMI in the year 2018. Our initial plan was to include a total of 18 hospitals in the survey. These were distributed across the 6 strata as follows: 3 tertiary

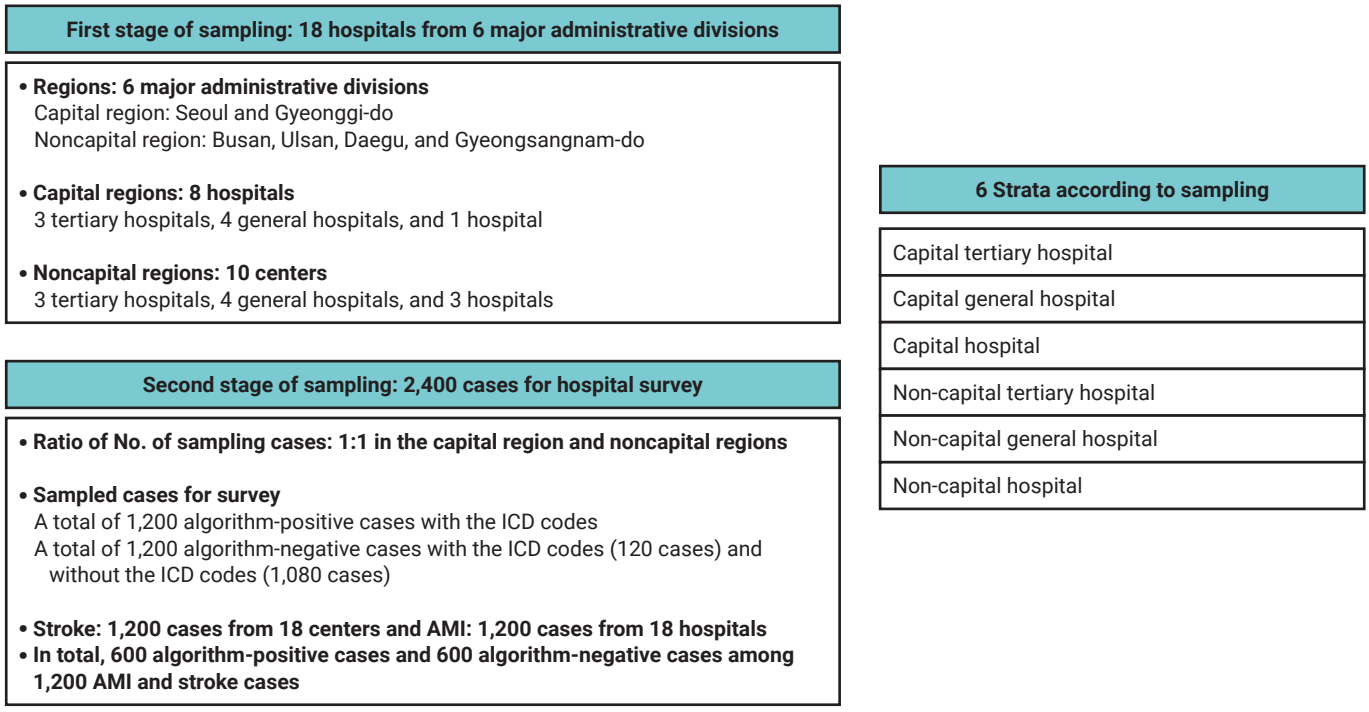

Figure 3. Two-stage stratified sampling method for the algorithm-positive and algorithm-negative groups.
Initially, 6 major administrative divisions and 18 hospitals were selected based on the feasibility of the survey. In the second stage, a specific number of cases from the 18 hospitals were determined for the survey. Ultimately, 6 strata were selected for the survey. The ratio of the numbers of tertiary hospitals to general hospitals was 8:10, based on the 8th Acute Stroke Quality Assessment Program (ASQAP) data, in which the number of tertiary hospitals and general hospitals was 42 and 356, respectively, with a sampling fraction of 20% in tertiary hospitals and 3% in general hospitals. The ratio of the sampled case volume was 1:1 between capital and non-capital regions and 6.5:2.5:1 between tertiary hospitals, general hospitals, and other hospitals, drawing from the 8th ASQAP data. For sampling cases for the hospital survey, stroke and acute myocardial infarction (AMI) algorithms were applied to cases with and without the International Classification of Diseases (ICD) codes. Cases with ICD codes were divided into 2 groups: the algorithm-positive group and the algorithm-negative group. In addition, algorithm-negative group without the ICD codes were identified by applying the algorithms to the 2018 National Health Insurance Service (NHIS) claims data.

hospitals, 4 general hospitals, and 1 other hospital from the capital region, and 3 tertiary hospitals, 4 general hospitals, and 3 other hospitals from the non-capital region (Figure 3; Table S1).

In the second stage, we determined the number of sampled cases with and without the ICD codes for stroke and AMI for each hospital and stratum. We employed the optimal allocation method [33,34] and adhered to these guidelines: (1) the ratio of sampled case volume was 1:1 between capital and non-capital hospitals, and 6.5:2.5:1 between tertiary, general, and other hospitals, based on data from the 8th ASQAP (Figure 3); (2) the ratio between algorithm-positive and algorithm-negative cases was 1:1 within each hospital; and (3) among the algorithm-negative cases in each hospital, 10% had the corresponding ICD codes, while 90% did not. As a result, our goal was to sample and survey a total of 1,200 algorithm-positive cases (600 for stroke and 600 for AMI) and 1,200 algorithm-negative cases (600 for stroke, including 60 with I60–I64 and 540 without, and 600 for AMI, including 60 with I21–I23 and 540 without) from the 18 participating hospitals (Figure 3; Table S1).

Hospital Survey

We enlisted 8 qualified reviewers for the survey with the support of the KDCA. Experts from the stroke and AMI working groups established the protocols for reviewing hospital records (Figure 1A). These reviewers underwent specialized training sessions to master how to evaluate sampled cases within the hospital settings. With the consent and support of the participating hospitals, the trained reviewers carried out the survey, consulting online with stroke and AMI experts as needed. The survey findings were scrutinized and confirmed by the respective stroke and AMI experts.

Validation of the Algorithms

The stroke and AMI algorithms were subjected to rigorous validation using the results derived from the hospital survey. The sensitivity, specificity, accuracy, PPV, and NPVs

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(NPV1 and NPV2) with their weighted values were estimated for each of the 6 strata by calculating a total sampling rate, taking into account the sampling structure and the corresponding sampling rates. We obtained 2 sampling rates. To calculate the first sampling rate, we divided the number of cases in selected hospitals by the total number of cases (admission episodes) from the 2018 NHIS claims data. We computed the second sampling rate by dividing the number of sampled cases from selected hospitals for the hospital survey by the number of cases in selected hospitals, which was used as a numerator to calculate the first sampling rate. The stratum-specific total sampling rate was obtained by multiplying these 2 sampling rates in each stratum (Figure 4).

During the hospital survey, the number of cases confirmed as either stroke/AMI or not were determined within each stratum across 3 groups: algorithm-positive cases with the ICD codes (used for PPV calculation), algorithm-negative cases with ICD codes (used for NPV1 calculation), and algorithm-negative cases without ICD codes (used for NPV2 calculation). By multiplying the obtained weights (the inverse of the total sampling rates) by the number of confirmed cases in each group within each stratum, we obtained the necessary values to calculate the weighted sensitivity, specificity, accuracy, PPV, and 1-NPV.

Estimation of Stroke and AMI Incidences

To obtain the number of incident stroke and AMI cases in the entire population, as well as to break these cases down by sex, we applied the algorithms on the 2018 NHIS claims

2-Stage simple sampling in each stratum

All episodes in each stratum from 2018 NHIS claims data → **Selected hospitals** → **Sampled cases for hospital survey**

Group	No. of cases
Algorithm-positive with the ICD codes	A
Algorithm-negative with the ICD codes	B
Algorithm-negative without the ICD codes	C

Group	No. of cases
Algorithm-positive with the ICD codes	$\sum D_i$
Algorithm-negative with the ICD codes	$\sum E_i$
Algorithm-negative without the ICD codes	$\sum F_i$

Group	No. of cases
Algorithm-positive with the ICD codes	$\sum g_i$
Algorithm-negative with the ICD codes	$\sum h_i$
Algorithm-negative without the ICD codes	$\sum i_i$

Sampling rates of each stratum

Sampling rate	Sampling rate 1	Sampling rate 2
Algorithm-positive with the ICD codes	$\sum D_i$ / A	$\sum g_i / \sum D_i$
Algorithm-negative with the ICD codes	$\sum E_i$ / B	$\sum h_i / \sum E_i$
Algorithm-negative without the ICD codes	$\sum F_i$ / C	$\sum i_i / \sum F_i$

Total sampling rates of each stratum

Sampling rate	Stratum-specific total sampling rate
Algorithm-positive with the ICD codes	$\sum g_i$ / A
Algorithm-negative with the ICD codes	$\sum h_i$ / B
Algorithm-negative without the ICD codes	$\sum i_i$ / C

Figure 4. Calculation of total sampling rates in each stratum.
In the intial stage of our sampling process, total cases were partitioned into 3 groups: (1) algorithm-positive cases with the International Classification of Diseases (ICD) codes, (2) algorithm-negative cases with the ICD codes, and (3) algoritm-negative cases without the ICD codes. Sampling rate 1 was determined by dividing the total number of cases in selected hospitals, after applying the algorithms and ICD codes, by the total number of cases derived from applying the algorithm and ICD codes to the 2018 National Health Insurance Service (NHIS) claims data. This calculation was made for each stratum. Sampling rate 2 was calculated by dividing the number of sampled cases for the hospital survey by the total number of cases in selected hospitals after implementing the algorithms in each stratum. The total sampling rate was calculated by multiplying sampling rate 1 by sampling rate 2. This provided a comprehensive measure of the sampling efficiency for the hospital survey, accounting for both the algorithm-based selection and the actual case sampling.

data and ascertained the number of cases within individual strata. The number of stroke and AMI cases within each stratum was computed by multiplying the stratum-specific weighted PPV by the number of algorithm-positive cases with ICD codes and the 1-NPV by the number of algorithm-negative cases with the ICD codes (NPV1) and without the ICD codes (NPV2). The resulting numbers were then added within each stratum. Summing up the numbers of stroke and AMI cases across all 6 strata provided an estimate of the incident case number of stroke and AMI in 2018 (Figure 5).

The incidence rate of stroke and AMI was calculated by dividing the total number of new stroke or AMI cases in 2018, including recurrent events, by the total person-time observed in 2018, as shown in the equation below [24–27].

$$\text{Incidence rate} = \frac{\text{No. of all occurrence of stroke or AMI in 2018}}{\text{Total person} - \text{Time of observation in 2018}}$$

The incidence proportion of stroke and AMI represents the proportion of individuals who developed stroke or AMI among the entire Korean population in 2018. For the incidence proportion, the numerator is the number of patients who experienced stroke or AMI in 2018, while the denominator is the total population at risk in mid-2018, as illustrated in the equation below [24–27]. Recurrent events were not counted.

$$\text{Incidence proportion} = \frac{\text{No. of patients with stroke or AMI in 2018}}{\text{Total population at risk in the middle of 2018}}$$

Finally, the age-and sex-standardized incidence rate and proportion, as well as the age-standardized rate and proportion, were estimated using the mid-year population of the Republic of Korea in 2005 as a benchmark. For age standardization, the WHO standard population in 2000 served as the reference.

Statistical Analysis

In this study, continuous variables were expressed as means and standard deviations, while categorical variables were presented as counts and percentages. The sensitivity,

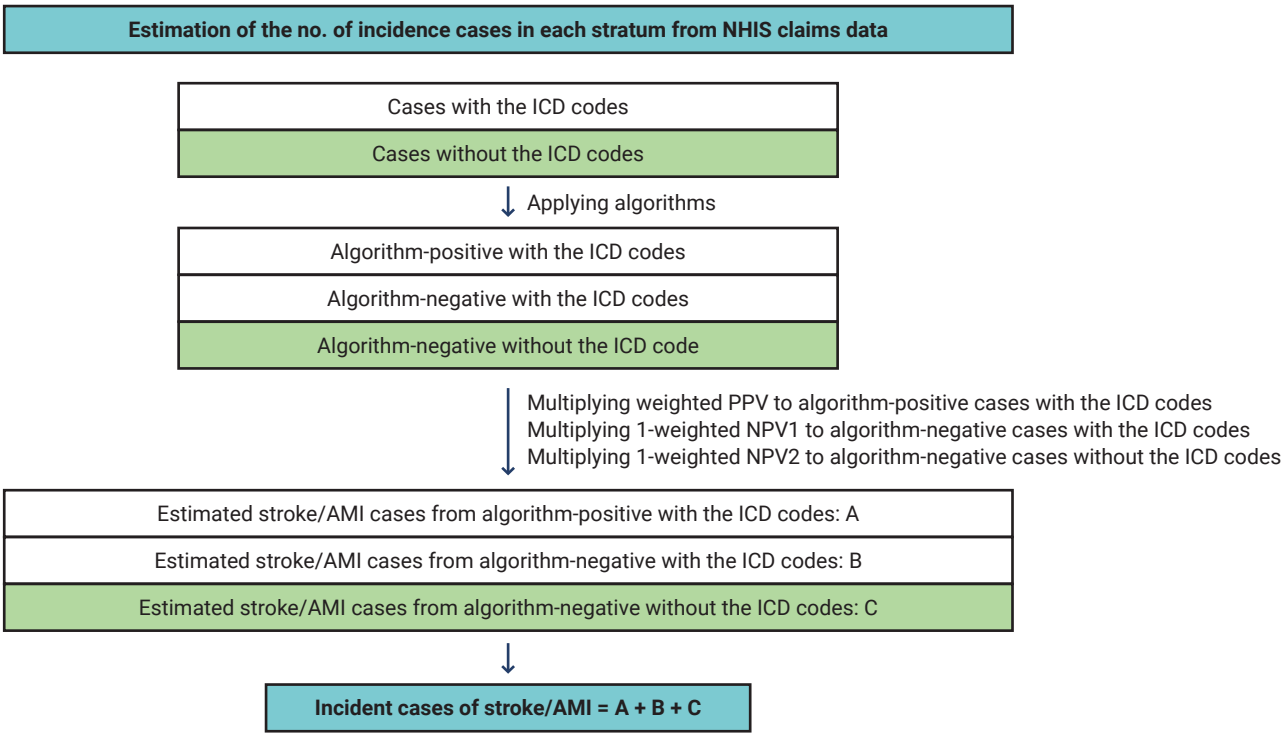

Figure 5. Estimation of the number of stroke and acute myocardial infarction (AMI) cases in each stratum.
Initially, we applied the algorithms to cases both with and without the International Classification of Diseases (ICD) codes to estimate the number of algorithm-positive and algorithm-negative cases. Subsequently, we computed the weighted values for each category. For algorithm-positive cases with the ICD codes, we multiplied the weighted PPV by the number of cases. For algorithm-negative cases with the ICD codes, we multiplied 1 minus the weighted NPV1 by the number of cases. Similarly, for algorithm-negative cases without the ICD codes, we multiplied 1 minus the weighted NPV2 by the number of cases. Finally, we derived the incident cases of stroke or AMI by adding these values (A, B, and C) calculated for each category. This approach allowed us to estimate the overall number of incident cases, taking into account the performance of the algorithm and the presence or absence of ICD codes in the data.
NHIS, National Health Insurance Service; PPV, positive predictive value; NPV, negative predictive value.

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specificity, PPV, and NPV values, as weighted, were provided for the algorithms identifying stroke and AMI. Additionally, 95% confidence intervals (CIs) for incidence rates and incidence proportions were calculated using either the normal approximation or gamma confidence limits when appropriate. All statistical analyses were conducted using SAS ver. 9.4 (SAS Institute Inc.).

Ethics Approval

The Institutional Review Board (IRB) of Seoul National University (IRB No: E-2104-135-1213, E-2109-031-1252, and H-2106-064-1225) approved this study. Additionally, the requirement for informed consent was waived by the IRB because of the retrospective nature of this study.

Results

Study Milestones and Timeline

This study spanned 1 year, running from April 2021 through March 2022, as detailed in Figure 6. While we were on track in the early study period, securing IRB approval, recruiting hospital record reviewers, and hosting their training sessions, challenges arose when it came to case selection for the hospital survey and securing collaboration from the targeted hospitals. These delays impacted our roster of reviewers, necessitating a fresh round of recruitment and training. Moreover, the time constraints that resulted from these delays put a squeeze on our hospital survey duration and our schedule for estimating epidemiological indices by applying the validated algorithms to the 2018 NHIS claims data. Despite these hurdles, we successfully validated our identification algorithms for stroke and AMI, and derived estimates on the epidemiological indices for 2018 in Korea.

Establishment of Epidemiological Indices, Definitions and Identification Algorithms

The stroke identification algorithm incorporated 23 key identifiers pertinent to acute stroke management, such as brain imaging, stroke unit care, reperfusion therapy, antithrombotic therapy, and rehabilitation. This algorithm was primarily based on a previously developed ischemic stroke identification algorithm (Figure 2A; Table S1) [13]. To ensure the detection of cases with hemorrhagic stroke in addition to ischemic stroke, a 2-tiered strategy was employed. Initially, the previously validated algorithm for ischemic stroke was put into action. Following this, another segment of the algorithm, which included the admission route, in-hospital mortality, length of hospitalization, and

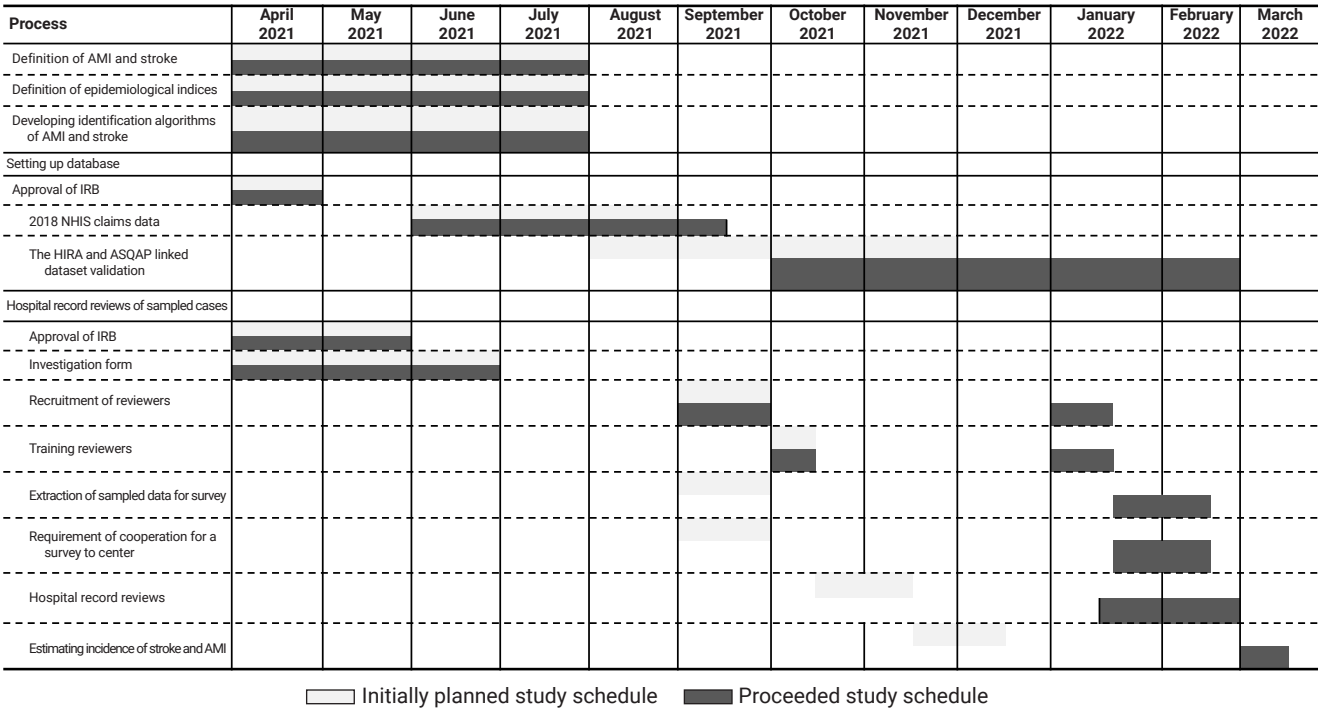

Figure 6. Study milestones.
AMI, acute myocardial infarction; IRB, Institutional Review Board; NHIS, National Health Insurance Service; HIRA, Health Insurance Review and Assessment Service; ASQAP, Acute Stroke Quality Assessment Program.

specific ICD codes, was used to detect hemorrhagic stroke cases. A preliminary survey conducted on 768 cases prior to the main hospital survey demonstrated that the PPV for identifying hemorrhagic stroke stood at an impressive 87.1%.

The AMI identification algorithm was tailored based on whether a hospital had the capacity to perform coronary angiography (CAG) (Figure 2B; Table S1). The categorization of being CAG-capable or not was based on whether the annual number of CAGs performed met or exceeded a threshold of 10. For CAG-capable hospitals, the algorithm included ICD codes, electrocardiogram (ECG) findings, cardiac enzyme levels (such as troponin I or troponin T), and CAG results. For CAG-incapable hospitals, the algorithm included ICD codes, ECG findings, and cardiac enzyme levels. A pilot study carried out before the main hospital survey, which involved a sample size of 757, found the PPV of the algorithm to be 73.3%.

Sampled Cases for the Hospital Survey

Due to the coronavirus disease 2019 (COVID-19)-related regulations and other logistical constraints, the planned hospital survey could not be conducted as initially intended. Instead of the originally planned 18 hospitals, only 14 hospitals participated in the survey. These consisted of 6 hospitals in the capital region (2 tertiary hospitals and 4 general hospitals) and 8 hospitals in the non-capital region (3 tertiary hospitals, 4 general hospitals, and 1 other hospital) (Table S2). The survey could not be carried out in the other hospital stratum of the capital region (Table S3).

There were also challenges in obtaining a sufficient number of algorithm-negative cases without ICD codes. This issue arose from communication gaps between the KDCA and the study teams, compounded by the tight timeline set for the study. As a result, we had to modify our approach to determining the sampling rates. We leaned on the NIHS-National Sample Cohort data regarding algorithm-negative cases without ICD codes. Further details regarding these adjustments can be found in Methods S1 and Figure S1.

In the hospital record review, we assessed 603 algorithm-positive cases and 483 algorithm-negative cases for stroke. Of these algorithm-negative cases, 54 cases had the ICD codes and 429 cases did not. For AMI, we evaluated 568 algorithm-positive cases and 546 algorithm-negative cases, with 60 of the latter having the ICD codes and 486 not having them (Table 1; Table S3). Through this review, we confirmed 578 cases (age, 72.3 ± 11.9 years; men, 51.9%) as stroke and 520 cases (age, 70.7 ± 10.9 years; men, 72.7%) as AMI (Tables 2 and 3). Of those confirmed as stroke, 88% involved ischemic stroke, while 98.5% of the AMI cases had undergone CAG. The in-hospital mortality rate was 6.2% for stroke cases and 6.3% for AMI cases.

Performance of the Algorithms

The results from the hospital survey indicated that the algorithm we developed to identify stroke had a sensitivity of 94.3% and a specificity of 88.6%. For AMI detection, the algorithm exhibited a sensitivity of 97.9% and a specificity of 90.1%. Table 4 details the PPV, NPV1, and NPV2 for both the stroke and AMI identification algorithms in each stratum.

The stroke and AMI identification algorithms had high PPVs, indicating a high proportion of true positive cases identified by the algorithms. However, the NPV1 for algorithm-negative cases with the ICD codes was found to be low for stroke, indicating a higher proportion of false negative cases in this category. Additionally, the PPVs for stroke and AMI were found to be lower in tertiary hospitals located in the capital region than in other types of hospitals in the capital region and the non-capital hospitals (Table 4).

Incidence of AMI and Stroke

By applying the algorithms to the 2018 NHIS claims database, we derived estimates of 150,837 incident stroke cases and 40,529 incident AMI cases. Men accounted for 55.7% of stroke cases and 66.8% of AMI cases. It is worth noting that due to the small sample size for NPV1 (comprising 10% of the total algorithm-negative group), we used the overall NPV1 (38.9% for stroke and 81.7% for AMI) instead of detailing results by each stratum to estimate incident cases. In 2018, the crude incidence rates of stroke and AMI were estimated to be 294.9 and 79.2 cases per 100,000 person-years, respectively. After standardization for age and sex, the incidence rates were calculated as 180.2 cases per 100,000 person-years for stroke and 46.1 cases per 100,000 person-years for AMI. Notably, the age-standardized incidence rates of both stroke and AMI were higher in men than in women (Table 5). When applying the individual NPV1 value obtained in each stratum instead of the overall NPV1, we found that the number of incident cases and the crude incidence rate was 152,241 cases and 297.6 per 100,000 person-years for stroke and 42,801 cases and 83.7 per 100,000 person-years for AMI, respectively.

Excluding recurrent cases, the estimated number of incident cases was 131,347 for stroke and 39,720 for AMI. The crude incidence proportions were 256.0 cases per 100,000 people for stroke and 77.4 cases per 100,000 people for AMI. After age and sex standardization, the incidence proportions were determined to be 154.1 cases per 100,000 people for stroke and 44.4 cases per 100,000 people for AMI. Similar to the incidence rates, the age-standardized

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Table 1. Results of hospital record reviews in stroke and AMI

Total centers	Stroke	Not a stroke	Total	AMI	Not an AMI	Total
Total cases	578	508	1,086	520	594	1,114
Algorithm-positive with the ICD codes	545	58	603	509	59	568
Algorithm-negative with the ICD codes	33	21	54	11	49	60
Algorithm-negative without the ICD codes	0	429	429	0	486	486
Cases in the capital region						
Tertiary hospitals	128	88	216	117	160	277
Algorithm-positive with the ICD codes	121	29	150	116	24	150
Algorithm-negative with the ICD codes	7	6	13	1	14	15
Algorithm-negative without the ICD codes	0	53	53	0	112	112
General hospitals	111	173	284	124	131	255
Algorithm-positive with the ICD codes	108	15	123	123	12	135
Algorithm-negative with the ICD codes	3	8	11	1	11	12
Algorithm-negative without the ICD codes	0	150	150	0	108	108
Cases in non-capital regions						
Tertiary hospitals	175	130	305	151	159	310
Algorithm-positive with the ICD codes	163	4	167	145	5	150
Algorithm-negative with the ICD codes	12	3	15	6	14	20
Algorithm-negative without the ICD codes	0	123	123	0	140	140
Non-capital regions						
General hospitals	144	97	241	116	132	248
Algorithm-positive with the ICD codes	135	8	143	114	7	121
Algorithm-negative with the ICD codes	9	4	13	2	10	12
Algorithm-negative without the ICD codes	0	85	85	0	115	115
Hospitals	20	20	40	12	12	24
Algorithm-positive with the ICD codes	18	2	20	11	1	12
Algorithm-negative with the ICD codes	2	0	2	1	0	1
Algorithm-negative without the ICD codes	0	18	18	0	11	11

AMI, acute myocardial infarction; ICD, International Classification of Diseases.

Table 2. Baseline characteristics of sampled cases of stroke for the hospital survey

Variable	Total (*n* = 1,086)	Stroke (*n* = 578)	Not a stroke (*n* = 508)	*p*
Age (y)	64.9 ± 19.0	72.3 ± 11.9	56.5 ± 21.9	< 0.001
Male	560 (51.6)	300 (51.9)	260 (51.2)	0.81
Admission routes				< 0.001
Direct visit	537 (49.4)	471 (81.5)	66 (13.0)	
During hospitalization	6 (0.6)	6 (1.0)	0 (0.0)	
Transfer	97 (8.9)	85 (14.7)	12 (2.4)	
Unknown	446 (41.1)	16 (2.8)	430 (84.6)	
Type of stroke				< 0.001
No stroke	508 (46.8)	0 (0)	508 (100.0)	
Ischemic stroke	511 (47.0)	511 (88.4)	0 (0)	
Hemorrhagic stroke	67 (6.2)	67 (11.6)	0 (0)	
History of MI	15 (1.4)	15 (2.6)	0 (0)	0.39
History of stroke	168 (15.5)	130 (22.5)	38 (7.5)	< 0.001
In-hospital mortality	38 (3.5)	36 (6.2)	2 (0.4)	< 0.001

Data are presented as mean ± standard deviation or *n* (%).
MI, myocardial infarction.

Table 3. Baseline characteristics of sampled cases of AMI for the hospital survey

Variable	Total (*n* = 1,114)	AMI (*n* = 520)	Not an AMI (*n* = 594)	*p*
Age (y)	61.3 ± 21.7	70.7 ± 10.9	53.0 ± 25.1	< 0.001
Male	691 (62.0)	378 (72.7)	313 (52.7)	< 0.001
Admission routes				< 0.001
Direct visit	484 (43.5)	408 (78.5)	76 (12.8)	
During hospitalization	15 (1.35)	13 (2.5)	2 (0.3)	
Transfer	107 (9.6)	99 (19.0)	8 (1.3)	
Unknown	508 (45.6)	0 (0.0)	508 (85.5)	
Chest pain	448 (40.2)	409 (78.7)	39 (6.6)	
Serum troponin I or T test	715 (64.2)	518 (99.6)	197 (33.2)	< 0.001
ECG	609 (54.7)	518 (99.6)	91 (15.3)	< 0.001
CAG	586 (52.6)	512 (98.5)	74 (12.5)	< 0.001
History of MI	79 (7.1)	47 (9.0)	32 (5.4)	0.001
History of angina	43 (3.9)	36 (6.9)	7 (1.2)	< 0.001
In-hospital mortality	42 (3.8)	33 (6.3)	9 (1.5)	< 0.001

Data are presented as mean ± standard deviation or *n* (%).
AMI, acute myocardial infarction; ECG, electrocardiogram; CAG, coronary angiography; MI, myocardial infarction.

Table 4. Validation of the identification algorithms for stroke and AMI after weighting

Region	Hospital	PPV (%)	NPV1 (%)	NPV2 (%)
Stroke				
Capital region	Tertiary hospital	80.7	53.3	100
	General hospital	87.8	69.4	100
Non-capital region	Tertiary hospital	97.6	20.0	100
	General hospital	94.4	31.2	100
	Other hospital	90.0	0	100
AMI				
Capital region	Tertiary hospital	77.3	95.0	100
	General hospital	91.1	91.7	100
Non-capital region	Tertiary hospital	96.7	80.0	100
	General hospital	94.2	83.3	100
	Other hospital	91.7	0	100

AMI, acute myocardial infarction; PPV, positive predictive value; NPV, negative predictive value.

incidence proportions of stroke and AMI were higher in men (Table 5).

Discussion

Our study showed that there were 150,837 incident stroke cases and 40,529 incident AMI cases across the Republic of Korea in 2018. When we excluded recurrent cases, the figures decreased to 131,347 for stroke and 39,270 for AMI. Compared to earlier studies in the Republic of Korea, which ranged from 73,501 to 130,025 [16,23,35], our estimated case number for stroke was much higher. Furthermore, our results exceeded those of the 2019 Global Burden of Disease study, which estimated 92,934 stroke cases in 2019 [2]. Likewise, the number of incident AMI cases in our study was also higher than those documented in previous studies in the Republic of Korea, which ranged from 15,893 to 25,531 [15,16,29], but was slightly lower than the figure reported in the first attempt to develop the national surveillance system (50,879 cases in 2004) [23]. Overall, our crude incidence rate and proportion exceeded those reported in previous Korean studies [15,16,23,35]. The marked rise in incident stroke and AMI cases compared to older studies might be attributed to the swift aging of the Korean population [36] coupled with an increased prevalence of traditional vascular risk factors stemming from the westernization of lifestyles [37]. While directly comparing our findings with these earlier studies can be complex due to variation in reference populations, disease definitions, and standardization methods, the upward trend in stroke and AMI case numbers we observed points to possible socioeconomic burdens linked to these health conditions.

The age- and sex-standardized incidence rates and proportions, based on the 2018 mid-year population of Korea,

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Table 5. Crude and age- and sex-standardized incidence of stroke and AMI in 2018

Variable	Stroke	AMI
Total incident cases	150,837	40,529
Total patients	131,347	39,720
Incidence rate		
Total (cases/100,000 person-year) (95% CI)		
Crude incidence rate	294.9 (293.4–296.4)	79.2 (78.5–80.0)
Age, sex-standardized incidence rate[a)]	180.2 (178.3–182.2)	46.1 (45.1–47.0)
Age-standardized incidence rate[b)]	175.6 (174.6–176.5)	46.0 (45.5–46.4)
Male (cases/100,000 person-year) (95% CI)		
Crude incidence rate	329.1 (326.9–331.3)	106.0 (104.8–107.3)
Age-standardized incidence rate)	196.3 (194.9–197.8)	62.2 (61.4–63.0)
Female (cases/100,000 person-year) (95% CI)		
Crude incidence rate	260.8 (258.9–262.8)	52.6 (51.7–53.5)
Age-standardized incidence rate[a)]	164.0 (162.7–165.4)	29.9 (29.3–30.3)
Incidence proportion		
Total (cases/100,000 people) (95% CI)		
Crude incidence proportion	256.0 (254.6–257.4)	77.4 (76.7–78.2)
Age, sex-standardized incidence proportion[a)]	154.1 (152.3–155.9)	44.4 (43.5–45.3)
Age-standardized incidence proportion[b)]	149.1 (147.1–151.1)	44.2 (43.8–44.7)
Male (cases/100,000 people) (95% CI)		
Crude incidence proportion	283.0 (280.9–285.0)	103.7 (102.5–105.0)
Age-standardized incidence proportion[a)]	166.4 (165.1–167.7)	60.2 (59.4–60.9)
Female (cases/100,000 people)		
Crude incidence proportion	229.2 (227.3–231.0)	51.2 (50.4–52.1)
Age-standardized incidence proportion[a)]	141.7 (130.5–142.9)	28.6 (28.1–29.1)

AMI, myocardial infarction; CI, confidence interval.
[a)]2005 Mid-year population in the Republic of Korea. [b)]2000 World Health Organization standard population.

were either comparable or slightly lower than those in the earlier Korean studies between 2004 and 2016 [15,16,23,35]. Our study's age-standardized incidence proportion of stroke (at 149.1 per 100,000 people using the 2000 WHO standard population) was similar to or slightly higher than the incidence of first-ever stroke observed in high-income countries [1,2,38–42], but lower than that in other Asian countries [43–45]. Regarding the age-standardized incidence proportion of AMI, our finding of 44.2 per 100,000 people was in line with the incidence of first-ever AMI in high-income Asian countries, but lower than the figures reported in Western countries [1,3,45–48].

We employed a 2-stage stratified sampling method to select cases for the hospital survey considering geographic location and hospital size in order to reflect the variability across different medical settings. This method is widely acknowledged for its effectiveness in estimating sensitivity, specificity, PPV, and NPV in national sample surveys while minimizing the standard error [30,31]. The hospital survey was conducted in 14 hospitals from 5 strata, involving a total of 2,200 cases (1,114 AMI and 1,086 stroke cases). Due to the restricted hospital access amid the COVID-19 pandemic and the constrained timeframe of our study, the numbers of cases and hospitals for the hospital survey were inadequate for comprehensive algorithm validation. In particular, the proportion of algorithm-negative cases with the corresponding ICD codes was only 10%, which limited the evaluation. This small sample size resulted in the variability of NPV1 across strata (Table 4) and the adoption of the overall NPV1 instead of the stratum-specific NPV1.

The NPV for cases classified as algorithm-negative with the ICD codes demonstrated lower values in our hospital survey. Specifically, the NPV1 for stroke was notably lower than that for AMI. This discrepancy could be largely attributed to the limited sampling of the algorithm-negative cases with ICD codes for the hospital survey: only 54 cases for stroke and 60 cases for AMI were surveyed (Table 1). This small sample size inherently limited the precision of our evaluation. Moreover, the difference can be attributed to the distinct disease characteristics and the nuances in ICD coding for AMI and stroke. The stroke ICD codes, in particular, clearly distinguish between acute and chronic stages of the condition. An analysis solely based on the ICD codes of the 2018 NHIS claims data revealed an overestimation in the reported numbers of both stroke and AMI compared to our estimates for incident stroke and AMI cases (Table

S4). This overestimation seemed to be more apparent for stroke than AMI. Additionally, clinical practices for AMI, including diagnostic testing and treatment, are generally less complex than those for stroke. The complexity of stroke care might have contributed to the lower NPV1 for stroke in our study.

The PPVs in the capital region were lower than those in the non-capital region, and tertiary hospitals in the capital region exhibited the lowest PPVs among the 5 strata. This reduced PPV might be explained by the fact that, in these strata, a considerable number of patients are hospitalized long after the acute phase has passed [49]. The influence of hospital size and geographic location on the PPV and NPV underscores the need for a more comprehensive hospital survey with a larger sample size to procure PPVs and NPVs based on region, size, and other characteristics of our healthcare system.

This study has several limitations. First, we relied on the overall NPV1 rather than the stratum-specific NPV1 due to the limited sample size of algorithm-negative cases with the ICD codes per stratum, which ranged from 1 to 20 for stroke and 2 to 15 for AMI. Second, the use of stratum-specific weighted values, derived from a hospital survey with limited case numbers from 14 hospitals across 5 strata in 6 administrative regions, may impede the generalizability of our findings. This sample might not have adequately represented the diversity of the entire country. Third, our inability to include non-hospitalized incident cases, including pre-hospitalization fatalities, is another limitation. Fourth, the short study period and the small number of patients surveyed restrict our capacity to evaluate the algorithm's accuracy in determining the exact incidence rate. Fifth, we could not estimate the lifetime first-ever incident cases due to inadequate historical data on previous stroke and AMI from the claims data.

However, the strengths of our study are noteworthy. To our knowledge, this is the first study to validate a developed algorithm, including PPV, NPV, sensitivity, and specificity, through an extensive hospital survey using a 2-staged sampling strategy for national representativeness. Unlike previous research focused mainly on the quality of acute care in AMI and stroke cases, our study uniquely estimated incidence rates and proportions using PPV and NPV stratification. Although few studies have specifically evaluated the validity of identification algorithms, our study demonstrated superior sensitivity, specificity, and PPV than those relying predominantly on ICD codes [17–23].

Additionally, our study highlights the feasibility of establishing a national surveillance system using claims data and identification algorithms for tracking the incidence of stroke and AMI. Such a system is invaluable for ongoing monitoring of these diseases and supporting nationwide epidemiological research. However, to implement effective 2-stage sampling for national hospital surveys and generate comprehensive national statistics, a unified platform for collaboration and streamlined data collection is imperative. Further studies with larger samples and a broader range of hospitals are essential to develop robust sampling strategies, ensuring accurate incidence estimates that reflect diverse healthcare settings.

Looking ahead, this study underscores the necessity for a national surveillance platform to minimize bias through a comprehensive hospital survey spanning the entire country based on claims data. It is important to continue adjusting and updating stroke and AMI identification algorithms based on extensive surveys with sample case numbers and study duration. Developing methods to estimate incident cases, including non-hospitalized ones and fatalities, is also crucial. Expanding the number and diversity of participating hospitals and allowing sufficient time for surveys will enhance the comprehensiveness and representativeness of our statistics. Moreover, the swift adaptation of the ICD-11 classification system is important for improving the accuracy of estimates [49]. Further research and the establishment of a well-organized platform for this surveillance system are essential steps forward.

Supplementary Material

Table S1. Key identifiers of AMI and stroke algorithms; **Table S2.** Planned number of cases for hospital survey; **Table S3.** Number of cases of completed hospital survey; **Table S4.** Case numbers of AMI and stroke solely based on the ICD codes obtained from the 2018 NHIS claims data; **Figure S1.** Modification of the calculation of sampling rates of each stratum for algorithm-negative cases without the International Classification of Diseases (ICD) codes; **Methods S1.** Supplementary method for calculating the sampling rate. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0248.

Notes

Ethics Approval

The Institutional Review Board (IRB) of Seoul National University (IRB No: E-2104-135-1213, E-2109-031-1252, and H-2106-064-1225) approved this study. Additionally, the requirement for informed consent was waived by the IRB because of the retrospective nature of this study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data
All data analyzed during this study are included in this published article. While the datasets are not publicly available, inquiries regarding access to the study data can be directed to the corresponding author.

Authors' Contributions
Conceptualization: HJK, JoL, HJB; Data curation: SEK, JP, JES, JiL; Formal analysis: TJK, HSL, SEK, JP, JES, JiL; Funding acquisition: HJB; Investigation: TJK, HSL, SEK, JYK, JP, JES, JiL; Methodology: TJK, HSL, SEK, JYK, JiL, JHC, HCK, DHS, HYL, BJK, WKS, JMP, SJL, KHJ; Project administration: JHH, JP, JoL, KH, HJK, HJB; Software: SEK, JP, JES, JiL; Supervision: HJK, SUK, YCH, HSK, JuL, HJB; Validation: TJK, HSL, SEK, JYK, JP, JES, JiL; Visualization: TJK, SEK, JP; Writing–original draft: TJK, HJB; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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Original Article

Impact of long COVID-19 on posttraumatic stress disorder as modified by health literacy: an observational study in Vietnam

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ABSTRACT

Objectives: The prevalence of posttraumatic stress disorder (PTSD) has increased, particularly among individuals who have recovered from coronavirus disease 2019 (COVID-19) infection. Health literacy is considered a "social vaccine" that helps people respond effectively to the pandemic. We aimed to investigate the association between long COVID-19 and PTSD, and to examine the modifying role of health literacy in this association.

Methods: A cross-sectional study was conducted at 18 hospitals and health centers in Vietnam from December 2021 to October 2022. We recruited 4,463 individuals who had recovered from COVID-19 infection for at least 4 weeks. Participants provided information about their sociodemographics, clinical parameters, health-related behaviors, health literacy (using the 12-item short-form health literacy scale), long COVID-19 symptoms and PTSD (Impact Event Scale-Revised score of 33 or higher). Logistic regression models were used to examine associations and interactions.

Results: Out of the study sample, 55.9% had long COVID-19 symptoms, and 49.6% had PTSD. Individuals with long COVID-19 symptoms had a higher likelihood of PTSD (odds ratio [OR], 1.86; 95% confidence interval [CI], 1.63–2.12; $p < 0.001$). Higher health literacy was associated with a lower likelihood of PTSD (OR, 0.98; 95% CI, 0.97–0.99; $p = 0.001$). Compared to those without long COVID-19 symptoms and the lowest health literacy score, those with long COVID-19 symptoms and a 1-point health literacy increment had a 3% lower likelihood of PTSD (OR, 0.97; 95% CI, 0.96–0.99; $p = 0.001$).

Conclusion: Health literacy was found to be a protective factor against PTSD and modified the negative impact of long COVID-19 symptoms on PTSD.

Keywords: Health literacy; Long COVID-19; Observational study; Posttraumatic stress disorder; Vietnam

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Introduction

Traumatic events occur throughout our lives, and most will fade over time [1]. However, in some instances, individuals may experience persistent flashbacks, nightmares, avoidance of trauma-related stimuli, changes in emotion and cognition, and disruptions in daily functioning following a traumatic event. When these symptoms persist for at least 1 month, the individual may be diagnosed with posttraumatic stress disorder (PTSD) [2]. The etiology of PTSD remains elusive, with theories suggesting a combination of biological factors (such as genetics and neurobiology), psychological determinants, and socio-environmental influences as contributing to the development of PTSD [2,3]. PTSD can also lead to a range of other mental health issues, including depression, anxiety, suicidal thoughts or behaviors, substance abuse, and eating disorders [4–6], and physical health problems, such as cardiovascular or metabolic diseases [7–9]. In severe cases, PTSD can last for decades if untreated [10,11].

The coronavirus disease 2019 (COVID-19) pandemic continues to have severe consequences on human life [12,13]. While most patients with COVID-19 recover fully, some individuals experience persistent symptoms after the acute phase of the infection, known as long COVID-19. Long COVID-19 is an umbrella term for symptoms that continue for at least 4 weeks following the initial onset of COVID-19 [14,15] and can last for years [16]. Globally, 43% of individuals who have had COVID-19 report experiencing some degree of long COVID-19 symptoms [17]. Previous studies have reported an

HIGHLIGHTS

- A high prevalence of posttraumatic stress disorder (PTSD) was found among people with long coronavirus disease 2019 (COVID-19).
- A higher health literacy score was associated with a lower likelihood of PTSD.
- People with long COVID-19 symptoms and a higher health literacy score were less likely to have PTSD.

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increased prevalence of symptoms such as anxiety, depression, cognitive impairment, memory loss, sleep disturbances, and PTSD in those affected by COVID-19 [18,19]. PTSD is prevalent in COVID-19 patients (15.45%), in the general population (17.24%), and even more so in healthcare workers (30.98%) [20]. The prevalence of PTSD is also high in survivors of previous pandemics; for instance, 43% of individuals who survived the Middle East respiratory syndrome exhibited clinical PTSD after 1 year, and 26% of severe acute respiratory syndrome survivors met the full criteria for a PTSD diagnosis after 30 months [21,22].

The COVID-19 pandemic has both direct and indirect effects on PTSD [23] and exacerbates other mental disorders [24]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters human cells and causes systemic inflammation, immune dysfunction, invasion, and brain damage, which may contribute to the development of PTSD [25]. Additionally, a variety of measures were implemented during the pandemic to prevent viral spread. Adherence to these measures varied according to factors such as age, educational background, trust in social institutions, and perceptions of social actors [26,27]. These interventions significantly altered daily life and have been associated with deteriorating mental health [28]. Although the pandemic does not conform to existing models or diagnostic criteria for PTSD, it is nonetheless considered a traumatic stressor [24]. Moreover, individuals may be affected by COVID-19 either through susceptibility to infection or the traumatic experience of witnessing the sudden death of a loved one, both of which can lead to significant stress with potential traumatic implications [29]. COVID-19 survivors have more severe PTSD symptoms than healthy people [30]. Furthermore, PTSD has been termed the "second tsunami" of the pandemic due to its chronic symptoms, impairment of social functioning, and increased risk of suicide [31]. In addition, PTSD causes unfavorable COVID-19 outcomes [32]. Therefore, assessing and monitoring PTSD symptoms in COVID-19 patients is crucial for mitigating the severe effects of long COVID-19 and enhancing mental well-being.

Promoting health literacy is a global public health goal in contemporary healthcare [33]. Health literacy is defined as the capacity to access, comprehend, appraise, and apply information pertaining to health and disease [34,35]. The COVID-19 pandemic led to an "infodemic" of misinformation and rumors from unreliable sources, which caused fear and panic in the community [36]. The importance of health literacy has become increasingly recognized and emphasized for both communities and individuals when dealing with emergencies such as the COVID-19 pandemic [37]. Health literacy can enhance people's understanding of COVID-19 symptoms, foster adherence to measures aimed at curbing the virus's spread (including hygiene practices, physical distancing, and behavioral changes), and support the uptake of COVID-19 vaccines [38,39]. Moreover, numerous studies have shown that individuals with adequate health literacy are less likely to suffer from mental health issues during the COVID-19 pandemic, such as depression, anxiety, and sleep disturbances [40–42]. However, research on the impact of health literacy on PTSD, particularly among patients with long COVID-19, remains limited.

We aimed to investigate the association between long COVID-19 and PTSD and examine the modifying role of health literacy on this association in people who had recovered from COVID-19 infection for at least 4 weeks. We hypothesized that health literacy could modify the negative impact of long COVID-19 on PTSD.

Materials and Methods

Study Design and Setting

We conducted a cross-sectional study through an online survey at 18 health centers and hospitals in various regions of Vietnam, spanning from December 2021 to October 2022. Data collection took place at hospitals, field hospitals, and Centers for Disease Control and Prevention (CDC) sites, encompassing 9 health facilities in the north (including 3 settings in Thai Nguyen Province, 1 hospital in Hai Duong Province, 4 settings in Hai Phong City, and 1 hospital in Hanoi City), 3 facilities in the central region (located in Quang Tri Province, Thua Thien Hue Province, and CDC Da Nang), and 6 facilities in the south (in Ho Chi Minh City and Can Tho City).

Investigators at each facility trained research assistants (undergraduate and postgraduate students, nurses, and doctors) on how to administer the survey questionnaires. These research assistants then reached out to patients who had recovered from COVID-19, using the contact lists provided by their respective health facilities, to invite them to participate in the survey. Upon agreeing, participants were sent a survey link that included an online consent form and the questionnaire via SMS. It took participants approximately 30 minutes to complete the survey.

Participants

Out of the 11,626 individuals who had recovered from COVID-19 and were invited from 18 facilities to participate in the study, 5,977 patients agreed and provided their consent. A total of 4,466 eligible patients were aged 18 to 85 years, were able to read and understand Vietnamese, and had recovered from COVID-19 infection for at least 4 weeks. Those with missing data related to health literacy

and PTSD measures were excluded from the survey (n = 3). Consequently, a final sample of 4,463 eligible participants was analyzed in this study.

Variables

Outcome variable

The impact of event scale-revised (IES-R) was utilized to evaluate symptoms of PTSD following unexpected exposure to traumatic stress over the past 7 days [43]. This questionnaire consists of 22 self-reported items, corresponding to the Diagnostic and Statistical Manual of Mental Disorders–4th Edition (DSM-IV) symptoms of PTSD with 3 subscales: intrusion, avoidance, and hyperarousal. The scale was validated among Vietnamese during the pandemic [44]. Each item was scored from 0 (not at all) to 1 (a little bit), 2 (moderately), 3 (quite a bit), and 4 (extremely). The total IES-R score ranged from 0 to 88, with a higher score representing more severe PTSD symptoms. The IES-R score was also classified into 4 levels, from normal (0–23) to mild or clinical concern PTSD (24–32), a moderately probable diagnosis of PTSD (33–36), and severe PTSD (≥37) [43]. The IES-R demonstrated a strong diagnostic capability for traumatic stress reactions, with an overall diagnostic accuracy of 0.88 at a cut-off score of 33, a sensitivity of 0.9, and a specificity of 0.82 [43]. Therefore, for the purposes of this study, we classified an IES-R score below 33 as indicating no PTSD, and a score of 33 or higher as indicative of PTSD symptoms.

Independent variables

In this study, the independent variables included sociodemographic characteristics (age, sex, marital status, educational attainment, healthcare workers, the ability to pay for medications, social status, family members with COVID-19 infection), clinical parameters (body mass index [BMI], number of COVID-19 infections, long COVID-19 symptoms, comorbidities), health-related behaviors (balanced meals, physical activity), and health literacy. Health literacy was evaluated as a potential effect modifier.

Sociodemographic characteristics and clinical parameters

Participants provided information on their age, sex (male or female), marital status (never married or ever married), level of education (high school or below versus college or above), status as healthcare workers (no or yes), ability to pay for medication (very or fairly difficult vs. fairly or very easy), social status (low, middle, or high), weight (kg), height (m), number of family members infected with COVID-19 (1 person vs. ≥2 people), number of COVID-19 infections (first time or more), and presence of comorbidities (0 vs. 1 or more). We calculated BMI using participants' weight and height (kg/m^2). BMI was categorized into 3 groups: underweight (less than 18.5 kg/m^2), normal weight (18.5 kg/m^2 to less than 23 kg/m^2), and overweight (23 kg/m^2 or greater).

Health-related behaviors

Health-related behaviors included whether meals were balanced (no vs. yes) and the physical activity of participants. The International Physical Activity Questionnaire–Short Form (IPAQ) is a widely used tool for assessing physical activity in adults [45,46]. We evaluated the participants' physical activity over the previous 7 days across 4 intensity levels: vigorous, moderate, walking, and sitting. We also recorded the number of days and minutes participants engaged in each activity. Metabolic equivalent of task (MET) values, expressed in minutes per week, were utilized to quantify overall physical activity [47]. Craig et al. [45] introduced the formula for the calculation of MET min/wk by multiplying the time spent on vigorous, moderate, walking, and setting activities by 8.0, 4.0, 3.0, and 1.0, respectively. The IPAQ has been validated in Vietnam [48].

Long COVID-19 assessment

According to the guidance from the National Institute for Health and Care Excellence, "long COVID-19" is defined as a condition characterized by signs and symptoms that persist for at least 4 weeks after the onset of a COVID-19 infection, with no other explanation [49]. People were classified as having or not having long COVID-19 symptoms based on whether they had any of the following symptoms: fever, cough, shortness of breath, altered sense of smell/taste, muscle soreness, fatigue/tiredness, sputum, chest pain, headache, sore throat, dizziness, confusion, …, diarrhea, nausea/vomiting [50].

Health literacy

To evaluate participants' health literacy, we utilized the 12-item Short-Form Health Literacy Questionnaire (HLS-SF12) [51]. This questionnaire has been validated and used in Vietnam [51]. Participants responded to the questions on a 4-point Likert scale from 1 (very difficult) to 2 (fairly difficult), 3 (fairly easy), and 4 (very easy). The health literacy score was standardized to a uniform number from 0 to 50 and presented as an index according to the formula:

$$\text{Index} = (\text{mean} - 1) \times (50/3)$$

The mean is the average value of the evaluation items, 1 is the lowest mean, 3 is the mean range, and 50 is the maximum selected value. Higher health literacy index scores indicate higher health literacy [52].

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Study Sample Size

The α level was 0.05, the power was 0.95, the estimated prevalence of PTSD in COVID-19 patients is 22.9% [53], and the prevalence of PTSD before the COVID-19 pandemic was 5.6% [54]. With these values, we used G*Power 3.1.9.7 (http://www.gpower.hhu.de/) to calculate the sample size for logistic regression [55]. Therefore, the required sample for analysis was 285 participants. In our study, the sample collected was 4,463 participants which was satisfactory for the statistical models.

Data Analysis

Categoral variables were expressed as frequency and percentage, while continuous variables were presented as mean and standard deviation. The chi-square and Mann-Whitney U tests were conducted to compare categorical and continuous variables. Simple and multiple logistic regression analyses were performed to identify potential factors associated with PTSD in individuals who had contracted COVID-19. For the multiple regression model, we included independent variables that had a p-value of less than 0.2 in the simple regression analysis [56]. We also conducted an interaction analysis to examine the potential modifying effect of health literacy on the relationship between long COVID-19 symptoms and PTSD, after adjusting for sociodemographic factors, other clinical parameters, and health-related behaviors. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using IBM SPSS ver. 20.0 (IBM Corp.) [57].

Ethics Statement

The study was reviewed and approved by the Institutional Ethical Review Committee of Hanoi School of Public Health in Vietnam (No: 400/2021/YTCC-HD3 and 45/2022/YTCC-HD3). Patients were informed about the study's purpose and provided consent to complete the survey questionnaire. The survey was conducted anonymously.

Results

Participants' Characteristics

Among the participants, 2,213 individuals (49.6%) exhibited symptoms of PTSD, as indicated by an IES-R score of 33 or higher. Of the 4,463 participants, 72.5% were aged between 18 and 39 years, 55.7% were female, 72.3% had a college education or higher, 82.5% were from a middle social status, and 28% worked in healthcare. Clinically, 97.6% had experienced their first COVID-19 infection, 55.9% reported at least 1 long COVID-19 symptoms, and 28.1% had at least 1 comorbid disease. The average health literacy score was 31.23 ± 8.54. Comparative analysis revealed that the prevalence of PTSD symptoms varied significantly across several demographics and clinical parameters. These included age, educational attainment, ability to pay for medication, social status, having a family member with a COVID-19 infection, presence of long COVID-19 symptoms, comorbidities, balanced diet, and physical activity ($p < 0.001$ for all), as well as sex ($p = 0.008$).

Additionally, there was a significant difference in the mean health literacy scores between participants with PTSD symptoms and those without ($p < 0.001$) (Table 1). However, the prevalence of PTSD did not significantly differ between healthcare workers and non-healthcare workers ($p = 0.392$) (Table 1).

Factors Associated with PTSD

Multiple logistic regression showed that respondents who were middle-aged (odds ratio [OR], 0.82; 95% confidence interval [CI], 0.69–0.97; $p = 0.019$), had a college degree or higher educational attainment (OR, 0.84; 95% CI, 0.72–0.99; $p = 0.033$), had a fairly or very easy ability to pay for medications (OR, 0.77; 95% CI, 0.67–0.88; $p < 0.001$), had high social status (OR, 0.59; 95% CI, 0.41–0.85; $p = 0.004$), had balanced meals (OR, 0.58; 95% CI, 0.5–0.66; $p < 0.001$), and more physical activities (OR, 0.53; 95% CI, 0.47–0.63; $p < 0.001$ for tertile 2 or OR, 0.58; 95% CI, 0.48–0.69; $p < 0.001$ for tertile 3) had significantly lower odds of PTSD than their counterparts (Table 2). Individuals with a higher health literacy score had a lower likelihood of PTSD (OR, 0.98; 95% CI, 0.97–0.99; $p = 0.001$). Those with 1 or more long COVID-19 symptoms had a higher likelihood of PTSD (OR, 1.86; 95% CI, 1.63–2.12; $p < 0.001$) than those without long COVID-19. Additionally, having at least 2 family members with COVID-19 infection was associated with a higher likelihood of PTSD (OR, 1.24; 95% CI, 1.06–1.45; $p = 0.008$) (Table 2).

Interaction between Long COVID-19 Symptoms and Health Literacy on PTSD

In comparison to individuals without long COVID-19 symptoms and the lowest health literacy scores, those with long COVID-19 and higher health literacy (1-score increment) had a lower likelihood of PTSD (adjusted OR, 0.973; 95% CI, 0.958–0.988; $p = 0.001$) (Table 3).

Discussion

The principal finding of this study was that health literacy moderated the adverse effects of long COVID-19 symptoms on PTSD. Among the 4,463 participants deemed eligible, 49.6% exhibited symptoms of PTSD, and 55.9% had experienced at least 1 long COVID-19 symptoms. Individuals between the ages of 40 and 59 who had at least a college degree, reported

Table 1. Sociodemographics, clinical parameters, health behaviors, health literacy, and PTSD of participants

Characteristic	Total	Non-PTSD (*n* = 2,250, 50.4%)	PTSD (*n* = 2,213, 49.6%)	*p*
Age (y)[a]				< 0.001[b]
18–39	3,234 (72.5)	1,657 (73.6)	1,577 (71.3)	
40–59	852 (19.1)	447 (19.9)	405 (18.3)	
60–85	377 (8.4)	146 (6.5)	231 (10.4)	
Sex				0.008[b]
Male	1,977 (44.3)	1,041 (46.3)	936 (42.3)	
Female	2,486 (55.7)	1,209 (53.7)	1,277 (57.7)	
Marital status				0.502[b]
Never married	1,852 (41.5)	945 (42.0)	907 (41.5)	
Ever married	2,611 (58.5)	1,305 (58.0)	1,306 (58.5)	
Educational attainment				< 0.001[b]
High school or below	1,238 (27.7)	521 (23.2)	717 (32.4)	
College or above	3,225 (72.3)	1,729 (76.8)	1,496 (67.6)	
Ability to pay for medications				< 0.001[b]
Very or fairly difficult	2,356 (52.8)	1,041 (46.3)	1,315 (59.4)	
Fairly or very easy	2,107 (47.2)	1,209 (53.7)	898 (40.6)	
Social status				< 0.001[b]
Low	551 (12.3)	228 (10.1)	323 (14.6)	
Middle	3,683 (82.5)	1,861 (82.7)	1,822 (82.3)	
High	229 (5.2)	161 (7.2)	68 (3.1)	
Healthcare workers				0.392[b]
No	3,215 (72.0)	1,608 (71.5)	1,607 (72.0)	
Yes	1,248 (28.0)	642 (28.5)	606 (28.0)	
Family members infected with COVID-19				< 0.001[b]
1 Person	938 (21.0)	536 (23.8)	402 (18.2)	
≥ 2 People	3,525 (79.0)	1714 (76.2)	1,811 (81.8)	
Body mass index				0.395[b]
Underweight	553 (12.4)	276 (12.3)	277 (12.5)	
Normal weight	3,490 (78.2)	1,749 (77.7)	1,741 (78.7)	
Overweight	420 (9.4)	188 (10.2)	195 (8.8)	
No. of COVID-19 infections				0.054[b]
First time	4,354 (97.6)	2,205 (98)	2,149 (97.1)	
More than 1 time	109 (2.4)	45 (2.0)	64 (2.9)	
Long COVID-19 symptoms				< 0.001[b]
No	1,968 (44.1)	1,187 (52.8)	781 (35.3)	
≥ 1	2,495 (55.9)	1,063 (47.2)	1,432 (64.7)	
Comorbidities				< 0.001[b]
None	3,207 (71.9)	1,699 (75.5)	1,508 (68.1)	
≥ 1	1,256 (28.1)	551 (24.5)	705 (31.9)	
Balanced meals[a]				< 0.001[b]
No	2,658 (60.6)	1,161 (53.2)	1,497 (67.6)	
Yes	1,727 (39.4)	1,022 (46.8)	705 (31.9)	
Physical activity (MET min/wk)[a]				< 0.001[b]
Tertile 1 (MET < 417)	1,115 (25.0)	395 (17.6)	720 (32.6)	
Tertile 2 (417 ≤ MET < 2,208)	2,230 (50.0)	1,236 (54.9)	994 (44.9)	
Tertile 3 (MET ≥ 2,208)	1,115 (25.0)	619 (27.5)	496 (22.4)	
Health literacy index	31.23 ± 8.54	32.76 ± 8.62	29.67 ± 8.18	< 0.001[c]

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

PTSD, posttraumatic stress disorder; COVID-19, coronavirus disease 2019; MET min/wk, metabolic equivalents of task in minutes per week.

[a]Missing cases. [b]Results of the chi-square test. [c]Results of Mann-Whitney U test.

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Table 2. Factors associated with PTSD

Factor	PTSD			
	Model 1		Model 2	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (y)				
18–39	Ref.		Ref.	
40–59	0.952 (0.818–1.107)	0.523	0.815 (0.686–0.967)	0.019
60–85	1.662 (1.336–2.068)	<0.001	0.942 (0.718–1.234)	0.664
Sex				
Male	Ref.		Ref.	
Female	1.175 (1.044–1.322)	0.008	1.038 (0.910–1.184)	0.58
Marital status				
Never married	Ref.			
Ever married	1.043 (0.926–1.175)	0.491		
Educational attainment				
High school or below	Ref.		Ref.	
College or above	0.629 (0.551–0.718)	<0.001	0.843 (0.721–0.987)	0.033
Ability to pay for medications				
Very/fairly difficult	Ref.		Ref.	
Fairly or very easy	0.588 (0.522–0.662)	<0.001	0.765 (0.669–0.875)	<0.001
Social status				
Low	Ref.		Ref.	
Middle	0.691 (0.576–0.829)	<0.001	0.931 (0.765–1.132)	0.473
High	0.298 (0.214–0.415)	<0.001	0.590 (0.411–0.847)	0.004
Healthcare workers				
No	Ref.			
Yes	0.945 (0.829–1.076)	0.392		
Family members infected with COVID-19				
1 person	Ref.		Ref.	
≥2 people	1.409 (1.218–1.629)	<0.001	1.238 (1.058–1.449)	0.008
Body mass index				
Normal weight	Ref.		Ref.	
Underweight	1.008 (0.843–1.206)	0.929	0.953 (0.783–1.159)	0.628
Overweight	0.871 (0.711–1.067)	0.181	0.949 (0.756–1.169)	0.579
No. of COVID-19 infections				
First time	Reference		Ref.	
More than 1 time	1.459 (0.992–2.147)	0.055	1.391 (0.920–2.105)	0.118
Long COVID-19 symptoms				
None	Ref.		Ref.	
≥1	2.047 (1.816–2.309)	<0.001	1.860 (1.633–2.120)	<0.001
Comorbidities				
None	Ref.		Ref.	
≥1	1.442 (1.264–1.644)	<0.001	1.124 (0.970–1.304)	0.121
Balanced meals				
No	Ref.		Ref.	
Yes	0.535 (0.473–0.605)	<0.001	0.575 (0.503–0.657)	<0.001
Physical activity, MET (min/wk)				
Tertile 1	Ref.		Ref.	
Tertile 2	0.441 (0.380–0.512)	<0.001	0.534 (0.456–0.626)	<0.001
Tertile 3	0.440 (0.371–0.521)	<0.001	0.575 (0.479–0.691)	<0.001
Health literacy index	0.957 (0.950–0.964)	<0.001	0.979 (0.970–0.987)	0.001

Model 1: simple logistic regression model; model 2: multiple logistic regression model.
PTSD, posttraumatic stress symptoms; OR, odds ratio; CI, confidence interval; ref., reference; COVID-19, coronavirus disease 2019; MET min/wk, metabolic equivalent of task in minutes per week.

Table 3. Interaction effect of health literacy and long COVID-19 symptoms on PTSD

	PTSD			
	Model 1		Model 2	
	OR (95% CI)	*p*	AOR (95% CI)	*p*
Without long COVID-19 and lowest HL index	Ref.		Ref.	
With long COVID-19 and lowest HL index	6.523 (3.984–10.681)	<0.001	4.440 (2.641–7.464)	<0.001
Without long COVID-19 and HL index, 1-score increment	0.982 (0.971–0.993)	0.001	0.992 (0.981–1.004)	0.198
With long COVID-19 and HL index, 1-score increment	0.961 (0.947–0.976)	<0.001	0.973 (0.958–0.988)	0.001

Model 1: simple logistic regression model; model 2: multiple logistic regression model after adjusted for age, sex, educational attainment, ability to pay for medications, social status, the family member with COVID-19 infection, BMI, number of COVID-19 infections, comorbidity, balanced meals, and physical activity.

COVID-19, coronavirus disease 2019; PTSD, posttraumatic stress symptoms; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio; HL, health literacy; ref., reference.

ease in affording medications, had a high social status, consumed balanced meals, participated in regular physical activity, and exhibited higher levels of health literacy were less likely to have PTSD.

In our study, the prevalence of PTSD symptoms among individuals with COVID-19 is 49.6%, a figure significantly higher than the 15.4% reported among frontline healthcare workers in Taiwan [58]. Although frontline healthcare workers are also susceptible to PTSD during the COVID-19 pandemic, Lu et al. included both participants with confirmed COVID-19 cases and those without, and utilized a different instrument for assessing PTSD, namely the IES-6. These differences in study design and methodology likely account for the higher prevalence of PTSD observed in our study.

When compared to young adults, middle-aged individuals were found to have a significantly lower likelihood of experiencing PTSD. In line with previous research, older adults also showed a lower prevalence of PTSD than their younger counterparts [20,59,60]. Notably, it was predominantly middle-aged people who exhibited the lowest PTSD scores [60]. Factors contributing to the increased risk of PTSD among young people included feelings of isolation, fear of death, uncertainty about the future, and concerns regarding the long-term effects of COVID-19 on health [24,61]. Furthermore, middle-aged individuals demonstrated better adherence to COVID-19 preventive guidelines than young adults [62]. Na et al. suggested that older adults possess greater resilience and more effective stress-coping mechanisms [63]. However, older adults tend to experience more severe COVID-19 symptoms, complicated long COVID-19 symptoms, and higher mortality rates, which exacerbate comorbidities related to COVID-19 [64]. The increased burden on physical health among older adults pose a heightened risk for mental health problems. These findings suggest that variations in the study populations may contribute to the observed differences in PTSD risk across age groups.

Individuals with a college degree or higher are less likely to experience PTSD compared to those with only a high school diploma or less. Higher education is linked to stable socioeconomic status, better coping mechanisms, improved problem-solving skills, and a more optimistic outlook, as well as a healthier lifestyle [65,66]. The financial and socio-political uncertainties resulting from the COVID-19 pandemic have been found to exacerbate PTSD symptoms [67]. Moreover, a diet rich in nutrients and regular physical activity contribute to maintaining good mental health and managing mental disorders [68]. We found that individuals who regularly consumed balanced meals and engage in physical activity were at a reduced risk of developing PTSD. When the body is under stress, increased corticosteroids and insulin can stimulate appetite, and a balanced, nutritious diet can help alleviate stress [69]. In contrast, diets high in calories, saturated fat, and sodium are more likely to be associated with negative moods [70]. Furthermore, physical activity has been shown to reduce inflammatory activity [71], lower the risk of cardiovascular disease [72], and enhance mood [73].

Our findings also showed that people who had family members with COVID-19 and those with long COVID-19 symptoms had an increased potential for PTSD. Individuals with family members with COVID-19 in the intensive care unit had a higher prevalence of PTSD symptoms [74]. The presence of COVID-19 within a household increases the risk of infection for other members, which in turn heightens anxiety over contracting the disease and concerns for the health of loved ones. Those who contract COVID-19 often experience stigma and discrimination from their communities, resulting in lower social support and more psychological distress than healthy individuals [75]. The impact of COVID-19 extends beyond the physical and mental health of the individual; it also places long-term burdens on their family members' daily lives [76]. After recovering from COVID-19, some patients face the possibility of reinfection

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with SARS-CoV-2 or suffer from long COVID-19 symptoms. Those with long COVID-19 symptoms are burdened with worries about the future, fears of reinfection, concerns about their ability to return to work, and in some cases, suicidal thoughts [77]. We found that individuals with long COVID-19 symptoms had a higher likelihood of PTSD.

In our study, patients with comorbidities had a nonsignificantly higher likelihood of PTSD. However, robust evidence indicates that patients who have comorbidities and contract COVID-19 are more likely to develop severe symptoms or complicated sequelae of COVID-19 [78]. The prevalence of mental disorders was found to be high among cancer patients during the COVID-19 pandemic [79], and the COVID-19 pandemic has exerted a negative impact on the mental health of patients with chronic diseases [80]. Furthermore, the healthcare system has focused on the treatment of COVID-19, leading to inadequate treatment, insufficient support, and increased psychological distress for cancer patients [81]. Further research is warranted concerning PTSD and mental health among individuals with cancer or chronic diseases after COVID-19 infection.

This study found that individuals with higher health literacy were less likely to develop PTSD. Studies on the relationship between health literacy and mental problems during COVID-19 have also yielded consistent results. Alatawi et al. [82] reported a negative association between PTSD symptoms and a higher level of health literacy. Higher levels of health literacy could mitigate fear, depression, and anxiety, while concurrently enhancing overall quality of life [83]. Additionally, people with long COVID-19 symptoms who had higher health literacy exhibited a lower likelihood of PTSD. Health literacy is not only essential during the COVID-19 pandemic, but it will also be critical and urgent in post-COVID health crises [84,85]. People with a high level of health literacy will be more aware of their COVID-19 status, better understand COVID-19 symptoms, more accurately identify behaviors to prevent infection, and more readily accept COVID-19 vaccination [39,86]. The COVID-19 pandemic highlights the importance of developing health literacy for personal and community self-protection [84,87]. People with long COVID-19 symptoms who have higher health literacy will apply healthy behaviors and appropriate coping mechanisms to stress and adjust to life.

The study has several limitations. First, it was unable to determine the cause-and-effect relationship between long COVID-19 and PTSD. Second, the data were collected through an online survey using convenience sampling, which, while appropriate for the COVID-19 context in Vietnam, may result in sampling bias. Third, we could not assess the likelihood of PTSD among healthcare and non-healthcare workers due to insufficient information regarding the types of hospitals where healthcare workers were employed during the COVID-19 outbreak. Additionally, participants subjectively evaluated their meals' nutritional balance, which calls for further research to investigate the link between nutrition and PTSD. Fifth, the use of the IES-R for PTSD assessment, without a clinical interview, could lead to an overestimation of PTSD prevalence. Finally, we were unable to evaluate the direct impact of severe COVID-19 infection on PTSD. Despite these limitations, the study's findings indicate that individuals who have recovered from COVID-19 infection for at least 4 weeks may benefit from early interventions if they exhibit PTSD symptoms, potentially alleviating these symptoms and improving their overall quality of life.

Conclusion

Individuals with a higher health literacy score had a lower likelihood of PTSD. Notably, health literacy played a modifying role by reducing the adverse effects of long COVID-19 symptoms on PTSD. Consequently, promoting health literacy in the general population, particularly among those infected with COVID-19, may help alleviate the psychological impact of the COVID-19 pandemic and attenuate its long-term consequences.

Notes

Ethics Approval

The study was approved by the Institutional Ethical Review Committee of Hanoi School of Public Health in Vietnam (No: 400/2021/YTCC-HD3 and 45/2022/YTCC-HD3) and performed in accordance with the principles of the Declaration of Helsinki. Patients were informed about the study's purpose and provided consent to complete the survey questionnaire. The survey was conducted anonymously.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

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

Authors' Contributions

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

Additional Contributions

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Original Article

Risk factors for SARS-CoV-2 transmission during a movie theater outbreak in Incheon in the Republic of Korea, November 2021: a retrospective study

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ABSTRACT

Objectives: We examined factors contributing to the transmission of an acute respiratory virus within multi-use facilities, focusing on an outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a movie theater in the Republic of Korea.
Methods: This retrospective cohort study involved a descriptive analysis of 48 confirmed cases. Logistic regression was applied to a cohort of 80 theater attendees to identify risk factors for infection. The infection source and transmission route were determined through gene sequencing data analysis.
Results: Of the 48 confirmed cases, 35 were theater attendees (72.9%), 10 were family members of attendees (20.8%), 2 were friends (4.2%), and 1 was an employee (2.1%). Among the 80 individuals who attended the 3rd to 5th screenings of the day, 35 became infected, representing a 43.8% attack rate. Specifically, 28 of the 33 third-screening attendees developed confirmed SARS-CoV-2, constituting an 84.8% attack rate. Furthermore, 11 of the 12 cases epidemiologically linked to the theater outbreak were clustered monophyletically within the AY.69 lineage. At the time of the screening, 35 individuals (72.9%) had received 2 vaccine doses. However, vaccination status did not significantly influence infection risk. Multivariate analysis revealed that close contacts had a 15.9-fold higher risk of infection (95% confidence interval, 4.37–78.39) than casual contacts.
Conclusion: SARS-CoV-2 transmission occurred within the theater, and extended into the community, via a moviegoer who attended the 3rd screening during the viral incubation period after contracting the virus from a family member. This study emphasizes the importance of adequate ventilation in theaters.

Keywords: Aerosols; Air microbiology; Cinema; Outbreaks; SARS-CoV-2; Ventilation

Introduction

In the early stages of the coronavirus disease 2019 (COVID-19) pandemic, the primary mode of transmission for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was considered to be respiratory droplets within a 2-meter radius of infected individuals [1,2]. Consequently, initial public health measures to control SARS-CoV-2 infection focused on social distancing [3–5]. However, reports of transmission occurring at greater distances from infected persons have emerged worldwide, suggesting airborne transmission as a major route and emphasizing the importance of ventilation over social distancing [6]. In the Republic of Korea, the critical role of indoor ventilation was increasingly recognized following large-scale infections in March 2021, which were thought to be due to airborne transmission in fitness and call centers [7–10]. In May 2021, the World Health Organization and the United States Centers for Disease Control and Prevention officially acknowledged the potential for airborne transmission of SARS-CoV-2 in indoor settings that are poorly ventilated and crowded for prolonged periods [11,12].

As the approach to quarantine measures evolved, the need for proper indoor ventilation in theaters was not adequately addressed. On November 1, 2021, the government of the Republic of Korea introduced the "living with COVID-19" strategy. This was prompted by the achievement of a 70% nationwide completion rate of the second dose of the SARS-CoV-2 vaccine (as of October 26, 2021), along with a gradual decrease in the number of confirmed cases in the country [13]. In response, major multiplex operators began to designate certain screens as "vaccine-pass" auditoriums. These screens were accessible to individuals who had received both vaccine doses or who could present a negative real-time polymerase chain reaction (RT-PCR) test result from within the previous 48 hours, excluding minors under 19 years old. Unlike a general auditorium, a vaccine-pass auditorium had no policy of maintaining an empty seat between audience members, and eating and drinking were permitted during the movie. However, after a SARS-CoV-2 infection outbreak in a cinema, a reassessment of the health and safety policies of theaters was initiated. As a result, beginning on December 6, 2021, all screens were restricted to allow entry only to vaccinated individuals, again excluding minors; additionally, the consumption of food, excluding water and other beverages, was banned [14].

The potential for respiratory virus transmission via droplets and aerosolized particles in enclosed indoor settings, often described by the 3 "Cs" (close contact, confined spaces, and crowded places), was acknowledged prior to the SARS-CoV-2 pandemic [15]. Nevertheless, documented instances of large-scale outbreaks of respiratory infections occurring in theaters are rare on a global scale. In the present case, not only were theatergoers attending the 3rd screening of the day directly exposed to the infection source, but attendees of the subsequent screening, theater employees, family members, and friends were also confirmed through genetic analysis to have contracted the same viral genotype. This case carries serious implications, highlighting the extensive reach of transmission beyond the initial group exposed in the theater and eventually impacting the wider community. Moreover, it underscores the critical need for prioritizing quarantine measures in theaters, where people exist in close quarters for prolonged periods, even without engaging in strenuous physical activity.

Therefore, the objective of this study was to identify the risk factors associated with SARS-CoV-2 transmission in theater settings. To achieve this, we formulated hypotheses that considered epidemiological elements—specifically, the pathogen, host, and environment—that contribute to disease occurrence. These hypotheses were then subjected to rigorous scientific scrutiny and analysis.

HIGHLIGHTS

- Our objective was to identify risk factors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission during an outbreak originating in a movie theater in November 2021.
- Next-generation sequencing analysis confirmed that attendees of the 3rd screening of the day shared a viral genotype not only with individuals from the subsequent screening with whom they had no direct contact, but also with family members and friends.
- Inadequate ventilation and audience proximity in the auditorium were key factors in the SARS-CoV-2 outbreak.
- Insights from this study may contribute to the improvement of multi-use facilities and highlight the importance of establishing safe environments within cultural venues, including theaters.

Materials and Methods

Outbreak Recognition

On November 3, 2021, an Incheon resident attended the 3rd screening of the day at a local theater. By November 6, 2021, she began to experience symptoms such as muscle pain and

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headaches, and SARS-CoV-2 infection was confirmed that day. While monitoring local SARS-CoV-2 incidence, the Incheon metropolitan government identified additional confirmed cases that corresponded with the theater name, the date of the visit, and the screening time of the initial confirmed patient who had attended the theater. In response, the government conducted RT-PCR tests on audience members who attended the 3rd screening, as well as on the theater staff, in accordance with the guidelines outlined in Article 18 of the Infectious Disease Prevention and Control Act [16]. When the number of confirmed cases reached 10, the situation was classified as a theater outbreak. To minimize community transmission, the testing was then extended to include individuals who had attended subsequent screenings. In addition, the Incheon metropolitan government and the local public health clinic conducted a preliminary field epidemiological investigation to evaluate the risk of transmission within the theater, adhering to the COVID-19 response guidelines [14].

Case Definition and Study Population

This investigation was conducted as a retrospective cohort analysis. Confirmed cases were identified as individuals with infection verified by RT-PCR, irrespective of the presence of clinical symptoms [14]. In the context of this outbreak, the patient with the earliest diagnosis date was considered the index case. The suspected primary case was identified as the confirmed case that presented with the earliest symptom onset, as established by the basic epidemiological investigation. Among the potential sources of infection linked to the suspected primary case, the source with the earliest onset of symptoms was designated as the preceding source of infection.

The case definition for this study included individuals who attended the 3rd to 5th screenings of the day as well as staff members working at the theater in Incheon on November 3, 2021. It also encompassed patients who came into contact with a confirmed theater-related case during the infectious period, spanning from 2 days before to 14 days after symptom onset, and who subsequently tested positive for the infection. By November 23, 2021, a total of 48 individuals, comprising audience members, theater staff, family members, and friends, fulfilled the case definition criteria. However, of the 92 audience members who attended the 3rd, 4th, and 5th screenings, only 80 were selected for the associated analysis of risk factors. The remaining 12 were excluded due to inconsistencies between the reservation information and the actual details of the attendees.

Epidemiological Investigation

On November 24, 2021, we conducted an extensive field epidemiological investigation in collaboration with the Korea Institute of Construction Technology, with the goal of examining facility-related risk factors.

First, we conducted a thorough assessment of the structural characteristics of the building housing the theater. The theater auditoriums occupy the 4th to 7th floors of a building with 7 underground and 7 above-ground levels. Specifically, auditoriums numbered 1 through 6 are found on the 4th floor, while auditoriums 7 through 14 are on the sixth floor. The outbreak took place in auditorium 13, which has a stadium-style layout and a seating capacity of 213 people. The index case was present at the 3rd of 5 screenings held that day, and the movie was approximately 3 hours long—double the length of a typical film.

The auditorium was equipped solely with natural ventilation, facilitated by 2 doors. The entrance was positioned on the left side at the rear of the seating area, while the exit was situated on the left side near the screen. This arrangement presented difficulties in providing adequate ventilation on the right side of the space.

During interviews with theater managers, we sought detailed information regarding the ventilation system. These managers reported that approximately 5 minutes separated the exit of an audience and the entry of the next group, during which time intensive ventilation was achieved by opening doors and introducing 100% outdoor air. However, the absence of recorded data from the heating, ventilation, and air conditioning (HVAC) systems posed a challenge in verifying specific operational details. We also reviewed the operation manual for the auditorium's automatic HVAC system. While the system's capacity was considered adequate for the size of the auditorium, the operational guidelines were not explicitly stated. Notably, the manual lacked specifics on the duration of operation and the proportion of outside air introduced during a movie's runtime. Furthermore, the air conditioning system was not providing cooling at the time of viral transmission, due to prevailing cool weather conditions.

Consequently, we inferred that inadequate indoor ventilation may have contributed to the risk of airborne transmission. Additionally, we contemplated the potential for short-range droplet transmission, given the low airflow speed [17].

Case Investigation

The Incheon metropolitan government compiled a list of individuals who were potentially exposed to the virus by utilizing a visitor management system known as "Safety Call for Entry Control." This system automatically logs the date and time of entry when a visitor dials the facility's unique number. Subsequently, text messages were

dispatched to these individuals, recommending that they undergo testing for SARS-CoV-2. Local public health clinics interviewed individuals who tested positive, generated basic epidemiological investigation reports, traced the contacts of confirmed cases, and uploaded collected information to the COVID-19 information management system of the Korea Disease Control and Prevention Agency (KDCA). This digital surveillance system enabled us to access information about confirmed cases and their contacts. Additionally, we obtained data on the SARS-CoV-2 vaccination status of the study population from the KDCA's COVID-19 vaccination management system. The Korea Institute of Construction Technology assessed the adequacy of ventilation and the patterns of airflow within the auditorium. To determine the genetic relationship of the virus found in confirmed cases, the KDCA's Division of Emerging Infectious Diseases conducted whole-genome sequencing on viral RNA extracted from nasopharyngeal swab samples that tested positive for SARS-CoV-2, with a cycle threshold of less than 30 for the E and N genes. The sequences were aligned using MAFFT ver. 7 (https://mafft.cbrc.jp/alignment/server/index.html) [18], and maximum likelihood phylogenetic trees were created using Fast Tree ver. 2.1.9 (http://www.microbesonline.org/fasttree) [19]. A total of 77 genome sequences were submitted to the Global Initiative on Sharing All Influenza Data (Supplementary Material 1).

Data Analysis

Descriptive statistics, presented as percentages, were utilized to examine differences in demographic, clinical, and epidemiological characteristics among the 48 confirmed cases identified during the epidemiological investigation of this outbreak. Risk factors for infection were examined using univariate and multivariate logistic regression analyses, with adjustments for potential confounding variables of SARS-CoV-2 infection among the 80 attendees of the 3rd to 5th screenings. In line with the Republic of Korea vaccination policy at the time, minors (those under 19 years old) were not eligible for vaccination. Consequently, we categorized the study population into 2 groups based on this age cutoff. Contacts were classified based on the criteria for determining contacts of individuals with airborne-transmitted tuberculosis during flights. Specifically, attendees seated in the same row as the index case, as well as those in the 2 rows in front of and the single row behind that individual, were designated as close contacts. All other attendees were deemed casual contacts [20,21]. R ver. 4.1.0 (The R Foundation) was used for statistical analysis, and an alpha level of 0.05 was employed to determine statistical significance. Values were presented along with 95% confidence intervals (CIs).

IRB/IACUC Approval

This study was reviewed and approved by the Institutional Review Board of the KDCA (No: KDCA-2023-05-04-PE-01) and was performed in accordance with the principles of the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for informed consent was waived.

Results

Descriptive Analysis

To understand the temporal pattern of the outbreak, we constructed an epidemic curve based on the symptom onset date for symptomatic patients and the confirmation date for asymptomatic cases. The curve indicated a single exposure outbreak with a propagated pattern, suggesting that infections occurred continuously through person-to-person transmission following shared exposure to a common vehicle during the movie screening on November 3, 2021. Within the group of epidemiologically linked confirmed cases, members of 1 household exhibited symptoms starting on November 4, 2021, representing the likely source of the infection. Furthermore, since symptoms began among the moviegoers 2 days after the screening, the minimum incubation period was considered to be 2 days (Figure 1).

A spot map was created to assess the spatial distribution of the outbreak. Audience members were seated in close proximity to each other without social distancing, predominantly in the rear seats. During the 3rd screening, most individuals identified as close contacts contracted the virus. In contrast, for the 4th screening, the outbreak was not confined to a particular area; rather, the confirmed cases were more uniformly dispersed throughout the venue (Figure 2).

Of the 48 confirmed cases, the majority were theater attendees, at 35 individuals (72.9%). They were followed by 10 family members (20.8%), 2 friends (4.2%), and 1 member of the janitorial staff (2.1%). At the time of diagnosis, 38 patients (79.2%) presented with symptoms, and 35 (72.9%) had completed secondary vaccination. The confirmed cases had potential contact points not only in the movie theater but also in their homes and schools (Table 1).

Hypothesis Formulation

Based on the results of the descriptive analysis, we established the following hypotheses concerning the cause of the outbreak.

First, the transmission of SARS-CoV-2 within the theater is believed to have originated from a primary case who, despite being asymptomatic, had contracted the virus through contact with a household member. Furthermore, the immune responses among individuals who had received a second vaccine dose were inadequate in preventing SARS-

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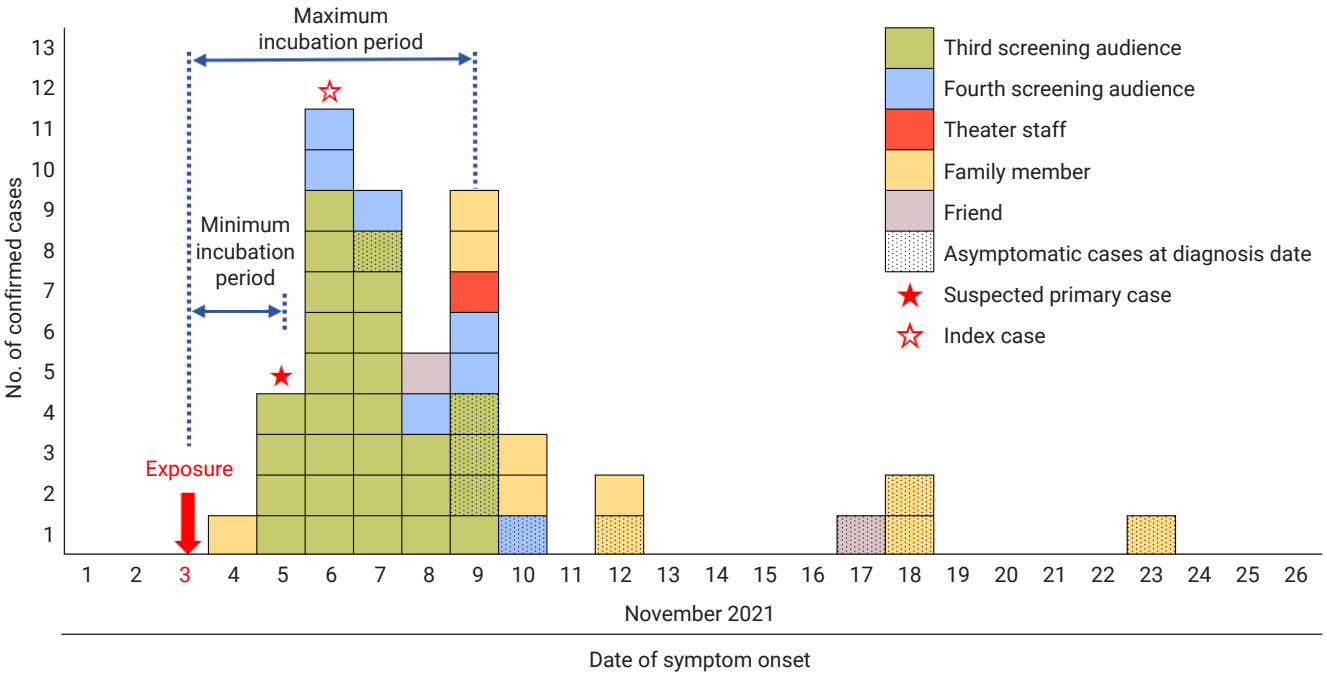

Figure 1. Epidemic curve of a severe acute respiratory syndrome coronavirus 2 outbreak originating in a movie theater within the Republic of Korea in November 2021 ($n = 48$).

CoV-2 infection. Finally, insufficient indoor ventilation in the auditorium substantially contributed to a widespread outbreak, which then extended into the local community.

Gene Sequencing Analysis

Next-generation sequencing analysis was performed on 77 cases—12 cases from the theater outbreak and 65 domestically identified positive cases from the same timeframe—to determine the source of infection and the molecular pathways of transmission. All cases were confirmed to have been caused by the SARS-CoV-2 Delta variant. Within the theater outbreak, 11 of the 12 cases were found to cluster within the AY.69 lineage (Figure 3). The remaining case from this outbreak was classified as belonging to the AY.122.5 lineage. Through genetic analysis and epidemiological investigation, a transmission chain was established (Figure 4). Experimental evidence confirmed that transmission occurred during a screening in the theater and continued into the subsequent screening, confirming airborne transmission. Infection also spread to family members and friends within the community.

Risk Factor Analysis

Among the 80 participants who attended the 3rd to 5th screenings, 35 individuals were confirmed to have contracted SARS-CoV-2 infection. This represents an attack rate of 43.8% within the theater (95% CI, 33.4%–54.7%). While only slight differences in attack rate were observed based on demographic characteristics such as sex and age, significant variations were observed with respect to epidemiological factors. Specifically, of the 33 participants who attended the 3rd screening, 28 tested positive, corresponding to an attack rate of 84.4% (95% CI, 69.1%–93.3%). In contrast, 7 of 37 attendees at the 4th screening were confirmed to have the infection, constituting an attack rate of 18.9% (95% CI, 69.1%–93.3%). No cases were detected among attendees of the 5th screening. Notably, all 6 individuals who consumed food during the movie screening tested positive for SARS-CoV-2. Regarding vaccination status, the attack rate was 70.0% (95% CI, 39.7%–89.2%) for those who had received 1 or fewer doses of the vaccine, compared to 40.0% (95% CI, 29.3%–51.7%) for those who had received 2 doses. With regard to proximity of exposure, the attack rate among casual contacts was 31.1 (95% CI, 20.9–43.6), while it was 84.2% (95% CI, 62.4%–94.5%) among close contacts.

To evaluate the risk factors associated with transmission, we conducted univariate and multivariate analyses, adjusting for potential confounders such as sex, age, screening attended, food consumption, vaccination status, and proximity of exposure. At the time of movie viewing, 35 individuals (72.9%) had received 2 vaccine doses, a status that was associated with 0.2-fold lower risk ratio (RR) (95% CI, 0.02–1.26) of infection compared to those with 1 or fewer doses, according to multivariable logistic regression analysis. However, this difference was not statistically significant. Significant

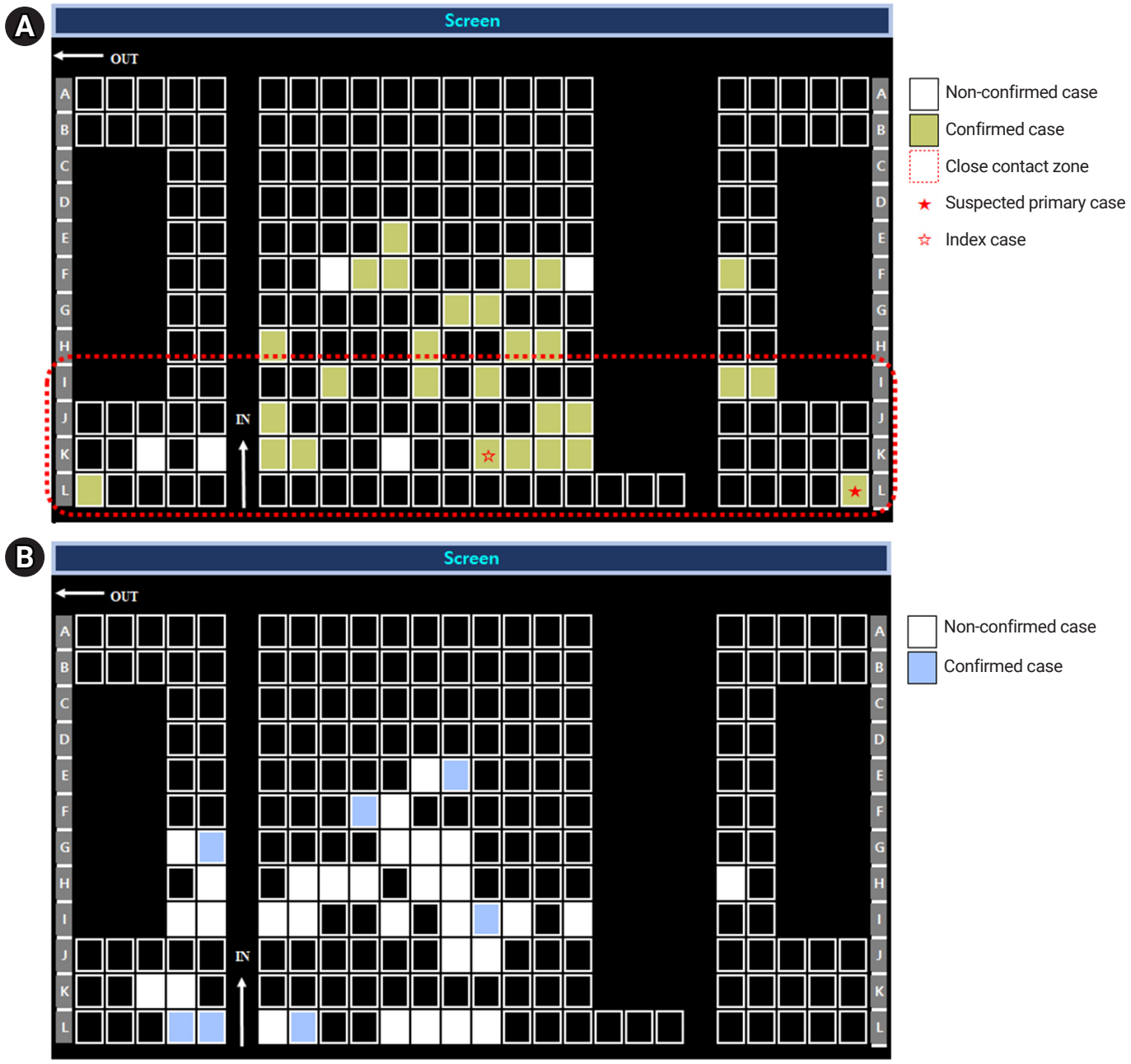

Figure 2. Seating distribution of confirmed severe acute respiratory syndrome coronavirus 2 cases in an outbreak originating in a movie theater in the Republic of Korea ($n=35$). (A) The 3rd screening, which took place on November 3, 2021, occurred from 17:10 to 19:56. (B) The 4th screening occurred on the same day, from 20:15 to 23:01.

associations were only detected in the analyses concerning screening attended and proximity of exposure. Relative to the 4th screening, the RR for the 3rd screening was 24.0 (95% CI, 7.4–93.8), while for close contacts, the RR was 11.8 (95% CI, 3.44–55.17) when compared to casual contacts. Moreover, the RR for close contacts rose to 15.9 (95% CI, 4.37–78.39) in the multivariable logistic regression analysis (Table 2).

Discussion

We investigated risk factors for acute respiratory virus transmission in multi-use facilities through a case study of a SARS-CoV-2 outbreak in a movie theater. Throughout the infectious period, from November 3, 2021 to November 23, 2021, we identified 48 confirmed cases linked to the theater. Specifically, during the 3rd screening—which included the

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Table 1. Characteristics of confirmed severe acute respiratory syndrome coronavirus 2 cases in a movie theater outbreak in the Republic of Korea, November 2021 (*n* = 48)

Characteristic	Confirmed case
Total	48 (100.0)
Sex	
Male	30 (62.5)
Female	18 (37.5)
Age (y)	
≤19	10 (20.8)
20–29	21 (43.8)
30–39	3 (6.3)
40–49	6 (12.5)
50–59	1 (2.1)
60–69	4 (8.3)
≥70	3 (6.3)
Classification	
Third screening audience	28 (58.3)
Fourth screening audience	7 (14.6)
Fifth screening audience	0 (0.0)
Theater staff	1 (2.1)
Family member	10 (20.8)
Friend	2 (4.2)
Symptoms	
Asymptomatic	10 (20.8)
Symptomatic	38 (79.2)
Vaccination status	
Unvaccinated or first vaccination	13 (27.1)
Second vaccination	35 (72.9)
Place of exposure	
Movie theater	36 (75.0)
House	10 (20.8)
Elementary school	1 (2.1)
Other[a)]	1 (2.1)

Data are presented as *n* (%).
[a)]Department store.

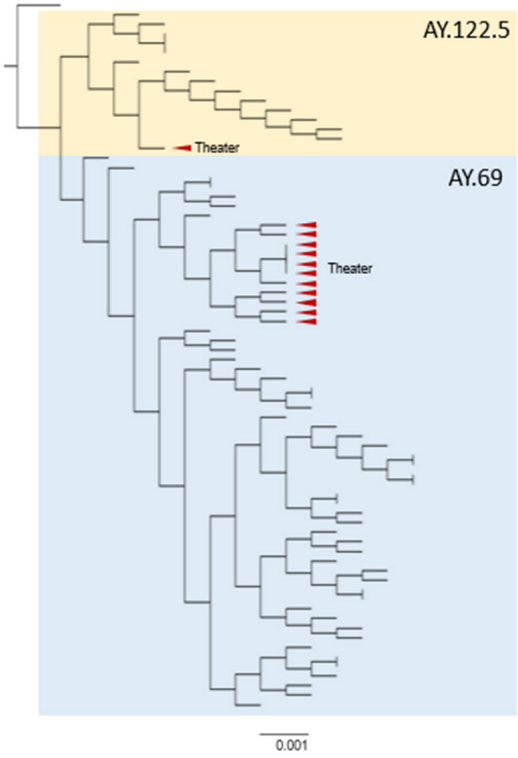

Figure 3. Phylogenetic analysis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome sequences associated with a SARS-CoV-2 outbreak originating in a movie theater in the Republic of Korea. Next-generation sequencing was performed on 77 confirmed cases, including 12 cases from this theater outbreak. The analysis revealed that 11 of the cases from the movie theater (indicated in red) belonged to lineage AY.69, while 1 case was classified as lineage AY.122.5. A maximum likelihood phylogenetic tree was constructed using FastTree ver. 2.1.9. The SARS-CoV-2 whole-genome sequences were submitted to the Global Initiative on Sharing All Influenza Data EpiCoV database (http://gisaid.org).

suspected primary case—28 of 33 attendees contracted the virus, representing an extremely high attack rate of 84.8%. Univariate analysis revealed that the risk of SARS-CoV-2 infection during the 3rd screening was 24 times greater than during the 4th screening, indicating direct airborne transmission. Moreover, after adjusting for potential confounding variables, individuals in close contact with the index case exhibited a RR approximately 15 times that of casual contacts. The extent of and risk factors identified in this outbreak underscore the potential for theater settings to become key hotspots for SARS-CoV-2 transmission, contributing to the spread of the virus.

In this study, we focused on exploring the transmission dynamics of SARS-CoV-2 within a theater environment by employing hypothesis testing methods. The results provide important insights into the various factors facilitating the rapid spread of the virus in a theater.

First, the high transmissibility of the Delta variant played a crucial role. Studies have shown that the Delta variant replicates more quickly within the body than non-variant viruses, with initial viral loads during infection reportedly 20 to 300 times higher than those seen in previous waves [22]. Notably, the Delta variant is characterized by increased viral shedding before the onset of symptoms and a higher volume of virus shed compared to earlier variants, which are critical factors in its rapid spread [23]. Given these virological characteristics, it is likely that the suspected primary case, who contracted the virus from a household contact, transmitted the virus in the theater during the pre-symptomatic incubation period [22,24,25].

Second, the diminished immune response observed among individuals exposed to the Delta variant is of considerable importance. The basic reproduction number (R_0) for the original coronavirus strain is 2.79, whereas the Delta variant

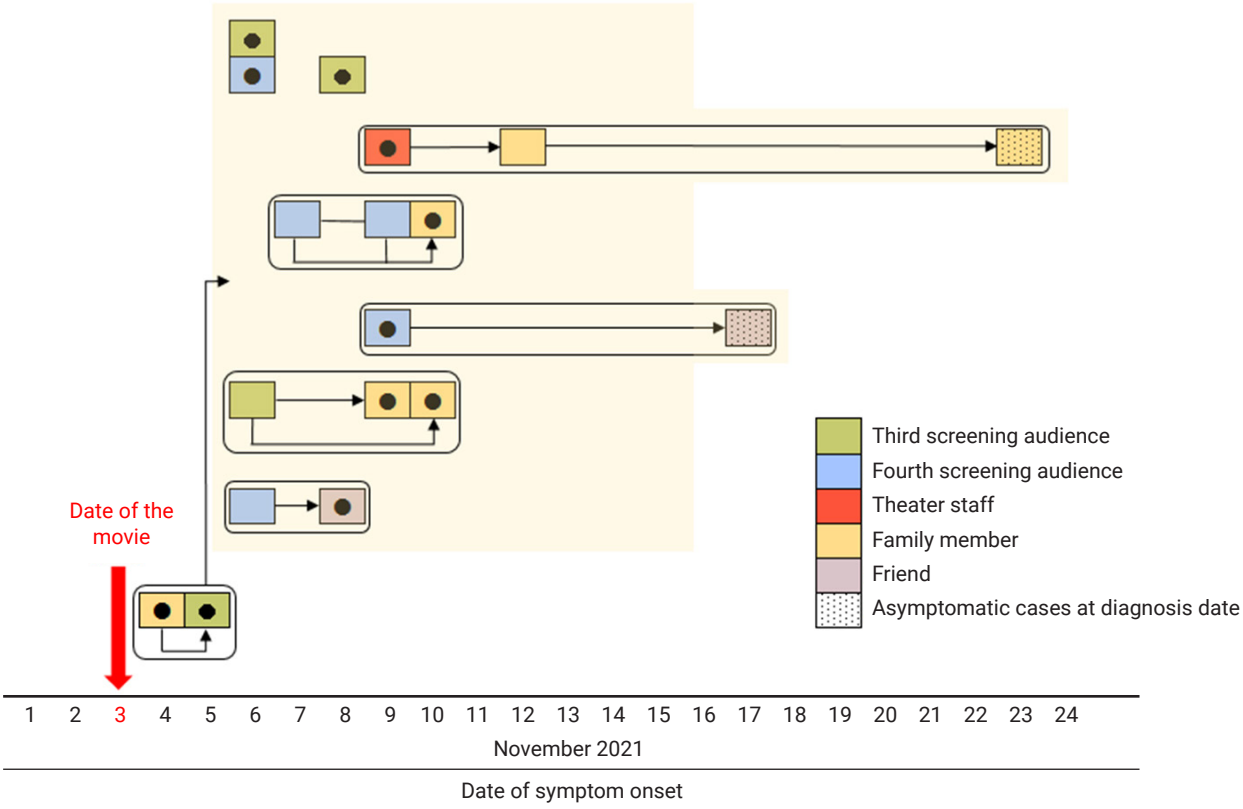

Figure 4. Transmission chain of a severe acute respiratory syndrome coronavirus 2 outbreak originating in a movie theater in the Republic of Korea, categorized by genotype. The black dots represent confirmed cases of the same genotype (AY.69) of the Delta variant (n = 11).

exhibits an R_0 of 5.08, reflecting its greater transmissibility [26]. Of the 80 theater attendees, 70 individuals (87.5%) had received a second vaccine dose and fell beyond the 14-day post-vaccination period; however, no significant difference was found in incidence risk based on vaccination status. Research from various sources, including the US Centers for Disease Control and Prevention, indicates that the protective effectiveness of vaccines developed against the original coronavirus strain is substantially diminished for the Delta variant [27]. Consequently, even the immunity conferred by a second vaccine dose appears inadequate in combating the Delta variant's more formidable epidemiological characteristics.

Lastly, inadequate ventilation that leads to indoor air contamination represents an additional risk factor. Next-generation sequencing analysis confirmed that the genotype present in attendees of the 3rd screening was also found in audience members at the following screening, as well as family and friends. This suggests transmission among attendees through shared contaminated air in the theater, with further spread to the community via direct and indirect contact. A multidisciplinary systematic review indicated that ventilation rates and airflow patterns are directly associated with the airborne diffusion of infectious agents [28]. In the present case, when the HVAC system is active in the auditorium, air is drawn out through exhaust vents located on the back walls of the seating area. This air is then purified by the system to remove contaminants before being supplied back into the room through ceiling vents. The ventilation and airflow investigation in this study revealed that the HVAC system was set to internal circulation mode, causing indoor air to diffuse throughout the entire auditorium within 3 minutes. If the filtration capacity of this system is inadequate, and if proper ventilation and expulsion of contaminated air are not achieved, the system faces the risk of recirculating contaminated air, leading to cross-contamination. Notably, the rear seats are particularly impacted by the HVAC system and are more vulnerable to air circulation issues due to the smaller space between the ceiling and the floor.

Transmission of SARS-CoV-2 can also occur through contact with surfaces or objects that have been contaminated with the virus, followed by touching the mouth, nose, or eyes with hands that have not been cleaned [2]. However, the virus does not exhibit high survival on surfaces [14]. Moreover,

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Table 2. Risk factors associated with confirmed severe acute respiratory syndrome coronavirus 2 cases in a movie theater outbreak in the Republic of Korea, November 2021 (*n* = 80)

Characteristic	Total	Confirmed cases	Attack rate	Crude risk ratio[a)]	Adjusted risk ratio[b)]
Total	80 (100.0)[c)]	35 (100.0)	43.8 (33.4–54.7)		
Sex					
Male	51 (63.8)	22 (62.9)	43.1 (30.5–56.7)	Ref.	Ref.
Female	29 (36.3)	13 (37.1)	44.8 (28.4–62.5)	1.1 (0.42–2.69)	1.7 (0.54–5.60)
Age (y)					
≤18	15 (18.8)	8 (22.9)	53.3 (30.1–75.2)	Ref.	Ref.
>19	65 (81.3)	27 (77.1)	41.5 (30.4–53.7)	0.6 (0.20–1.93)	1.0 (0.17–7.13)
Screening attended					
Third	33 (41.3)	28 (80.0)	84.8 (69.1–93.3)	24.0 (7.4–93.8)	NA
Fourth	37 (46.3)	7 (20.0)	18.9 (9.5–34.2)	Ref.	Ref.
Fifth	10 (12.5)	0 (0.0)	0.0 (0.0–27.8)	NA	NA
Food consumption					
No	74 (92.5)	29 (82.9)	39.2 (28.9–50.6)	Ref.	Ref.
Yes	6 (7.5)	6 (17.1)	100.0 (61.0–100.0)	NA	NA
Vaccination status					
Unvaccinated or first vaccination	10 (12.5)	7 (20.0)	70.0 (39.7–89.2)	Ref.	Ref.
Second vaccination	70 (87.5)	28 (80.0)	40.0 (29.3–51.7)	0.4 (0.09–1.42)	0.2 (0.02–1.26)
Proximity of exposure					
Casual contact	61 (76.3)	19 (54.3)	31.1 (20.9–43.6)	Ref.	Ref.
Close contact	19 (23.8)	16 (45.7)	84.2 (62.4–94.5)	11.8 (3.44–55.17)	15.9 (4.37–78.39)

Data are presented as *n* (%) or % (95% confidence interval).
Ref., reference; NA, not available.
[a)]The univariable model was adjusted for matching factors, including sex, age, screening attended, food consumption, vaccination status, and proximity of exposure. [b)]The multivariable model was adjusted for all variables incorporated within it, with the exceptions of screening attended and food consumption.
[c)]A total of 12 people were excluded from the study: 1 individual in the 3rd screening, 5 in the 4th screening, and 6 in the 5th screening.

the risk of transmission via fomites is deemed to be low due to widespread adherence to personal hygiene practices, including mask-wearing and hand hygiene [29].

This study had several limitations. First, we were unable to confirm whether the index case and the suspected primary cases were infected with the same viral genotype, due to the lack of a specimen for genetic analysis from the index case. Second, the omission of 12 attendees from the 3rd to 5th screenings because of incomplete personal information may have contributed to an overestimation of the attack rate within the theater. Third, this case reflects an outbreak influenced by a variety of epidemiological factors; therefore, it should not be generalized as a definitive instance of airborne transmission in movie theaters overall. Moreover, the potential contribution of mask usage to transmission cannot be dismissed, as no survey has been conducted to assess the mask-wearing rate and the types of masks used by audiences. Given the ongoing COVID-19 pandemic, theaters, as popular cultural venues, have implemented rigorous control and management measures. Consequently, it is crucial to also develop comprehensive infection prevention protocols for these settings. This necessitates a quantitative analysis of the dynamic properties of respiratory viruses, which underscores the need for additional research in the future.

Conclusion

In summary, this study substantially contributes to an understanding of the mechanisms of acute respiratory infection spread in multi-use facilities. This was specifically accomplished by analyzing the risk factors for SARS-CoV-2 transmission in a theater. The combined effects of the "3 C" elements heightened the risk of transmission, with the Delta variant's high transmissibility and inadequate immunity among contacts further accelerating the spread. Additionally, poor ventilation and suboptimal operation of the HVAC systems led to increased indoor air pollution, enabling wider virus dissemination. These findings underscore the need for a range of management strategies and infection control protocols to improve the safety of multi-use facilities. In particular, they emphasize the importance of ensuring that cultural venues, such as theaters, are safe environments. Future research should be focused on analyzing the effects of ventilation and sanitation measures through simulations, with the goal of developing practical guidelines applicable to real-world settings.

Supplementary Material

Supplementary Material 1. The SARS-CoV-2 whole-genome sequences have been submitted to the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (http://gisaid.org). The GISAID accession IDs for these sequences can be found in the Supplementary Data. This supplementary information also details the process for calculating the sampling rate. The supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0269.

Notes

Ethics Approval

This study was reviewed and approved by the Institutional Review Board of the KDCA (No: KDCA-2023-05-04-PE-01) and was performed in accordance with the principles of the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for informed consent was waived.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This investigation was conducted as part of a primary initiative of the Center for Epidemiological Investigation and Analysis at the KDCA. The name of this initiative is "Reinforcement of epidemiological investigation capacity and establishment of a research base" (grant No: 6231-3335-210-01).

Availability of Data

The SARS-CoV-2 whole-genome sequences have been submitted to the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (http://gisaid.org). The GISAID accession IDs for these sequences can be found in Supplementary Material 1.

Authors' Contributions

Conceptualization: HYL, YJP, SEL; Data curation: HNY, IHK, JSN; Formal analysis: HYL, YJP; Funding acquisition: MY, SEL; Investigation: HNY, HYL, YJP, JY, SB, IHK, JSN, MY; Methodology: HYL, YJP, SEL, JY, SB, IHK, EJK; Project administration: YJP, SEL; Resources: YJP, SEL, EJK; Software: HYL, IHK, JSN; Supervision: YJP, SEL, EJK; Visualization: HYL, IHK, JSN; Writing–original draft: HYL, YJP; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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Original Article

Prevalence, multidrug resistance, and biofilm formation of *Vibrio parahaemolyticus* isolated from fish mariculture environments in Cat Ba Island, Vietnam

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ABSTRACT

Objectives: *Vibrio parahaemolyticus* is a major foodborne pathogen in aquatic animals and a threat to human health worldwide. This study investigated the prevalence, antimicrobial resistance, antimicrobial resistance genes (ARGs), and biofilm formation of *V. parahaemolyticus* strains isolated from fish mariculture environments in Cat Ba Island, Vietnam.
Methods: In total, 150 rearing water samples were collected from 10 fish mariculture farms in winter and summer. A polymerase chain reaction assay was used to identify *V. parahaemolyticus*, its virulence factors, and ARGs. The antimicrobial resistance patterns and biofilm formation ability of *V. parahaemolyticus* strains were investigated using the disk diffusion test and a microtiter plate-based crystal violet method, respectively.
Results: Thirty-seven *V. parahaemolyticus* isolates were recovered from 150 samples. The frequencies of the *tdh* and *trh* genes among *V. parahaemolyticus* isolates were 8.1% and 21.6%, respectively. More than 90% of isolates were susceptible to ceftazidime, cefotaxime, and chloramphenicol, but over 72% were resistant to ampicillin, tetracycline, and erythromycin. Furthermore, 67.57% of isolates exhibited multidrug resistance. The presence of ARGs related to gentamicin (*aac(3)-IV*), tetracycline (*tetA*) and ciprofloxacin (*qnrA*) in *V. parahaemolyticus* isolates was identified. Conversely, no ARGs related to ampicillin or erythromycin resistance were detected. Biofilm formation capacity was detected in significantly more multidrug-resistant isolates (64.9%) than non-multidrug-resistant isolates (18.9%).
Conclusion: Mariculture environments are a potential source of antibiotic-resistant *V. parahaemolyticus* and a hotspot for virulence genes and ARGs diffusing to aquatic environments. Thus, the prevention of antibiotic-resistant foodborne vibriosis in aquatic animals and humans requires continuous monitoring.

Keywords: Antimicrobial resistance; Biofilm; Multidrug resistance; *Vibrio parahaemolyticus*; Virulence factor

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Introduction

Aquaculture environments are considered to be reservoirs of various biological pollutants, including infectious agents and antimicrobial resistance genes (ARGs) [1]. ARGs, which are contaminants of emerging concern [2], encode antimicrobial resistance and play an essential role in the process of resistance prevalence and proliferation [3]. In the environment, ARGs can be transferred from one microorganism to another, including from non-pathogenic to pathogenic species, through horizontal gene transfer. Pathogenic microorganisms that carry ARGs within aquaculture settings represent one of the most significant global health hazards, with millions of individuals succumbing to foodborne and waterborne diseases annually [4].

Vibrio parahaemolyticus is a halophilic marine bacterium that belongs to the family Vibrionaceae and is widely distributed in brackish water and marine environments [5]. This bacterium is a facultative human pathogen and is responsible for approximately 25% of all foodborne diseases closely associated with the consumption of raw seafood [6]. The ingestion of contaminated raw fish and shellfish, or seafood that has not been sufficiently heat-treated, can lead to symptoms such as diarrhea, abdominal pain, vomiting, chills, and low-grade fever in humans [7]. The significance of infections caused by this pathogen is increasing in public health due to a steady rise in its incidence worldwide over recent decades. *V. parahaemolyticus* is considered an emerging pathogen with a global distribution. Its infectious capability is mediated by various virulence factors, including hemolysin, urease, 2 type III secretion systems, and 2 type VI secretion systems, as well as the formation of persister cells [8]. To survive during infections and in seafood, *V. parahaemolyticus* cells may also form biofilms, which shield the cells from host defenses and external factors, including antibiotics, thereby contributing to antimicrobial resistance.

Antibiotics are widely used in aquaculture for both disease prevention and the treatment of infections potentially caused by *V. parahaemolyticus* [4]. However, the long-term use of antibiotics, particularly at incorrect dosages, can lead to the development of antimicrobial resistance in marine bacteria, including *V. parahaemolyticus* [4]. Moreover, antibiotics present in aquatic products and seafood can be ingested by animals and humans, potentially leading to the development of resistance in microorganisms that cause diseases in both animals and humans. This resistance can diminish the effectiveness of these antibiotics in treating infections.

To date, hundreds of *V. parahaemolyticus* strains have been isolated from seafood and shrimp farming environments in Vietnam [9–11]. However, no previous study has investigated the molecular resistance mechanisms and biofilm formation of *V. parahaemolyticus* in marine fish environments in Vietnam. Cat Ba Island is a significant marine fish production area and a well-known tourist destination in the northeast of Vietnam. To mitigate outbreaks caused by the consumption of raw or undercooked fish contaminated with *V. parahaemolyticus*, and to prevent potential risks to human health, it is necessary to monitor the prevalence of *V. parahaemolyticus* in fish mariculture rearing water. Additionally, identifying antimicrobial resistance and ARGs, virulence factor genes, and biofilm formation is of paramount importance. The aim of this study was to improve our understanding of the current state of antimicrobial resistance in the marine environment and the risks posed by the virulence factor genes and ARGs of *V. parahaemolyticus*, which can cause foodborne diseases in aquatic animals and humans.

HIGHLIGHTS

- This is the first study on *Vibrio parahaemolyticus* isolated from fish mariculture in Vietnam.
- 8.1% and 21.6% of isolates harbored the *tdh* and *trh* genes.
- A high percentage of isolates exhibited resistance to ampicillin (86.5%), erythromycin (75.7%), and tetracycline (73.0%), and more than 67% of isolates were multidrug-resistant.
- A close correlation between the resistance phenotypes of *V. parahaemolyticus* and genotype was identified for ciprofloxacin and tetracycline.
- Biofilm formation ability was detected in more multidrug-resistant isolates (64.9%) than non-multidrug-resistant isolates (18.9%).

Materials and Methods

Sampling

The seawater samples were collected following the method described by Zhao et al. [12], with slight modifications. In brief, water from the fish mariculture environment was collected from a depth of 10 to 20 cm below the seawater surface. A total of 150 samples were collected from 10 grouper mariculture farms in Lan Ha Bay (Cat Ba Island), Hai Phong (Figure S1 and Table S1), during the survey period between February 2021 and August 2021.

Bacteria Isolation

V. parahaemolyticus was isolated as described by Mok et al. [13]. Briefly, 5 mL of seawater was added to a flask containing 95 mL of peptone water with 1.5% NaCl and then incubated at 28 °C for 24 hours. Subsequently, 100 µL aliquots were spread on thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Merck) and incubated for 48 hours at 28 °C. Colonies that grew on TCBS were characterized by their color, shape, and size. The blue colonies were subsequently inoculated onto CHROMagar medium (Titan). Purple presumptive colonies were further confirmed through biochemical and polymerase chain reaction (PCR) assays.

Genomic DNA Extraction and Identification of *V. parahaemolyticus*

Colonies of each suspected isolate were cultured in TSB medium (Merck) supplemented with 1.5% NaCl at 28 °C. After 24 hours of incubation, 1.5 mL of the bacterial culture was harvested by centrifugation at 13,000 rpm for 5 minutes. The total genomic DNA from each suspected isolate was then extracted using the Favorgen Tissue Genomic DNA Extraction Mini Kit (Favorgen), following the manufacturer's instructions. *V. parahaemolyticus* was identified by a PCR assay using *toxR*-specific primers [14]. The genomic DNA of *V. parahaemolyticus* from the collection of the Department of Fisheries, University of Agriculture and Forestry, Hue University was used as a positive control, while nuclease-free water was used as a negative control.

The presence of virulence genes was identified using the primers listed in Table S2. PCR reactions were performed in PTC200 thermal cyclers (Marshall Scientific). Each 25-µL reaction mixture contained 12.5 µL of MyTaq PCR master mix (Bioline), 1 µL of DNA template (50 ng/µL), 1 µL of each primer (10 pmol), and 9.5 µL of distilled water. The PCR program was as follows: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, an annealing temperature of 50 to 60 °C for 30 seconds (varying based on the primer used), and extension at 72 °C for 1 minute, with a final elongation step at 72 °C for 5 minutes. PCR products were visualized by agarose gel electrophoresis using Safe DNA stain (AAT Bioquest).

Antimicrobial Susceptibility Profiling and PCR Detection of ARGs

The antimicrobial susceptibility of each *V. parahaemolyticus* isolate was assessed using the disc diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) M45 guidelines [15]. Initially, colonies from each isolate were suspended in 1% NaCl and adjusted to match the 0.5 McFarland standard. This suspension was then spread onto Mueller-Hinton agar plates (HiMedia). Subsequently, discs impregnated with 9 antimicrobial agents (Mast Diagnostics)—ampicillin (10 µg), ciprofloxacin (5 µg), kanamycin (30 µg), gentamicin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), ceftazidime (30 µg), and ceftiofur (30 µg)—were placed equidistantly on the surface of the agar. The plates were then incubated at 28 °C for 18 to 24 hours. *Escherichia coli* ATCC 25922 served as the quality control strain. The resistance profile of *V. parahaemolyticus* was determined by measuring the zones of inhibition, in accordance with the CLSI M45 guidelines for *Vibrio* spp. and the CLSI M100 guidelines [15,16]. The multiple antibiotic resistance (MAR) index was calculated using the formula a/b, where "a" is the number of antibiotics to which resistance was detected in a single isolate, and "b" represents the total number of antibiotics tested [17].

Concurrent with antimicrobial resistance testing, genotype screening was performed using PCR assays to detect the presence of ARGs for ampicillin, gentamicin, ciprofloxacin, erythromycin, and tetracycline. The primers for the targeted ARGs and the PCR conditions are detailed in Table S2.

Biofilm Formation Assay

The biofilm production capability of *V. parahaemolyticus* isolates was assessed using a previously described method [18], with some modifications. In brief, overnight cultures of *V. parahaemolyticus* grown in TSB with 1.5% (w/v) NaCl at 30 °C were diluted to an optical density at 600 nm (OD600) of 0.1. Then, 150 µL of the diluted cell suspension was aliquoted in triplicate into 96-well plates (SPL Life Science). After 24 hours of incubation, non-adherent cells and culture medium were removed, and the attached cells were washed 3 times with a 2 mM $CaCl_2$/$MgCl_2$ buffer. The cells were then stained with 0.01% crystal violet (Merck) for 15 minutes. Subsequently, the plates were washed 3 more times with the 2 mM $CaCl_2$/$MgCl_2$ buffer. To facilitate the measurement of absorbance, 70% ethanol was added to each well before the contents were transferred to a new 96-well plate. Absorbance was measured at a wavelength of 540 nm. The criteria for interpreting the biofilm formation capacity of *V. parahaemolyticus* were as follows, based on a previous report [19]. Briefly, OD > 4×ODc means strong biofilm formation; 2×ODc < OD ≤ 4×Odc, moderate biofilm formation; ODc < OD ≤ 2×Odc, weak biofilm formation; and negative biofilm formation when OD < ODc. ODc refers to the control measurement, which was performed in a microtiter plate without cells.

Statistical Analysis

The statistical analysis was carried out using IBM SPSS ver.

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20.0 (IBM Corp.). The significance of differences in biofilm formation, the prevalence of *V. parahaemolyticus*, and the distribution of ARGs was examined using the chi-square test. A p-value ≤ 0.05 was considered as significant.

Results

Prevalence of *V. parahaemolyticus* in Fish Farming Water

The distribution of *V. parahaemolyticus* in farming water is presented in Table 1. In total, 51 suspected *V. parahaemolyticus* isolates were obtained from a total of 150 samples, using TCBS agar and CHROMagar. These isolates were further verified by a PCR assay (Figure 1A) with *toxR* primers, confirming that 37 of them were *V. parahaemolyticus*. Of these, 11 isolates were collected from 76 samples obtained in the winter, while 26 were identified from 74 samples obtained in the summer. Consequently, the isolation rate of *V. parahaemolyticus* was significantly higher in the summer (35.1%) than in the winter (14.5%) ($p < 0.05$, chi-square test).

Table 1. The prevalence of *Vibrio parahaemolyticus* in water samples

Type of samples	No. of samples	No. of isolates (%)	X^2/p*
Sampled in winter	76	11 (14.5)	8.613/0.003
Sampled in summer	74	26 (35.1)	
Total samples	150	37 (24.7)	

*$p < 0.05$.

Detection of Virulence-Related Genes

The presence of the *tdh* and *trh* genes in all 37 *V. parahaemolyticus* isolates was examined. The results, as shown in Figure 1 and Table 2, indicated that 3 (8.1%) isolates were positive for the *tdh* gene and 8 (21.6%) isolates were positive for the *trh* gene. However, none of the isolates carried both the *tdh* and *trh* genes. Additionally, biofilm-associated genes were highly prevalent, with 83.8% of isolates harboring the *VP0952* gene, and the *VP0950* and *VP0962* genes were present in 81.08% of isolates.

Antimicrobial Resistance Pattern of the *V. parahaemolyticus* Isolates

The antibiotic resistance profile of 37 isolates against 9 antibiotics is presented in Table 3. None of the 37 isolates exhibited resistance to ceftazidime, cefotaxime, or chloramphenicol. A lower resistance rate was observed for ciprofloxacin (18.9%, 7/37), kanamycin (13.5%, 5/37), and

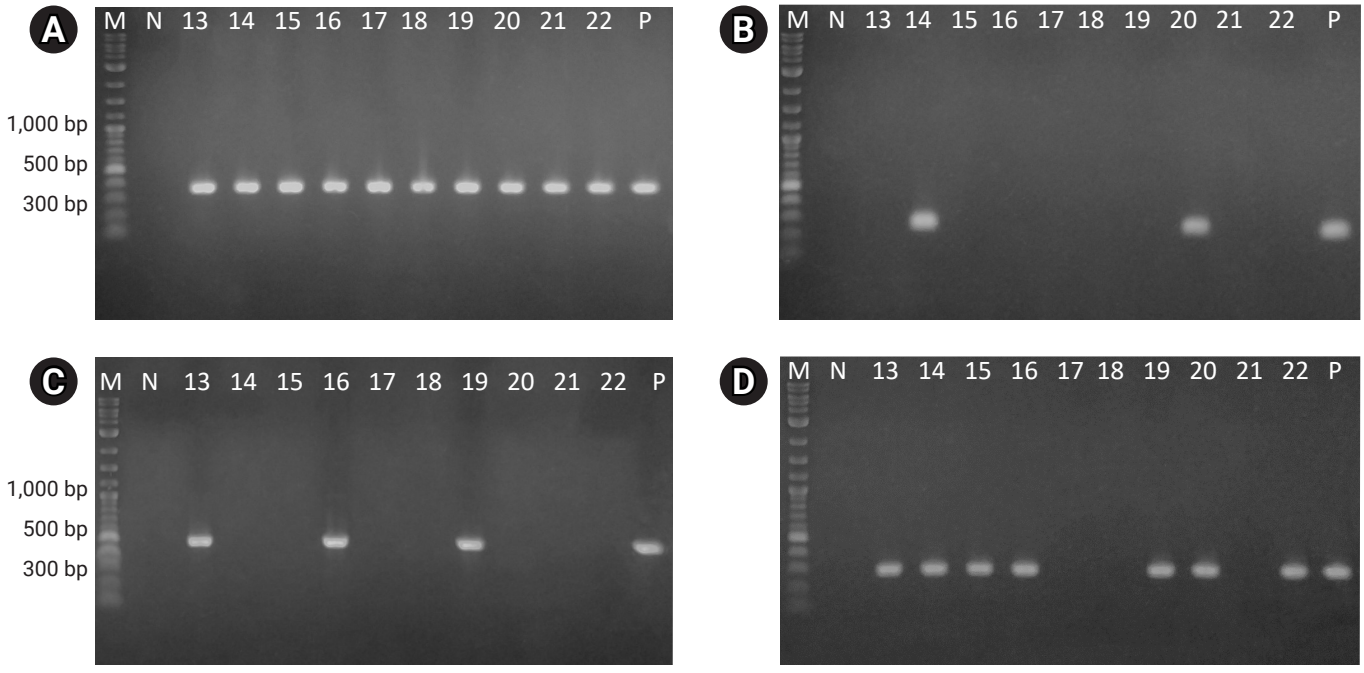

Figure 1. A representative agarose gel for *Vibrio parahaemolyticus*-specific polymerase chain reaction detection by *toxR* primers (A) and for the detection of virulence genes: *tdh* (B), *trh* (C), and *VP0950 ompK* (D). Lane M, 1 kb DNA ladder plus marker (Biolabs, NEB); lanes 13–22: sample number; lane N, negative control, lane P, positive control.

Table 2. The distribution of virulence genes, multidrug resistance profile, and biofilm formation of *Vibrio parahaemolyticus* isolates

Isolate name	MARI	Resistance phenotype	Biofilm formation	*toxR*	*tdh*	*trh*	*VP0950*	*VP0952*	*VP0962*
CB1	0.333	AMP TCN ERY	Moderate	+	–	–	+	+	+
CB2	0.333	AMP KAN TCN	Weak	+	–	–	+	+	+
CB3	0.333	CIP TCN ERY	Weak	+	–	–	+	+	+
CB4	0.222	AMP TCN	Moderate	+	–	+	+	+	+
CB5	0.333	CIP GEN ERY	Moderate	+	–	–	+	+	+
CB6	0.333	AMP TCN ERY	Weak	+	–	–	+	+	+
CB7	0.556	AMP CIP KAN TCN ERY	Moderate	+	–	–	+	+	+
CB8	0.222	AMP TCN	Weak	+	–	–	+	+	+
CB9	0.222	AMP TCN	Weak	+	–	–	+	+	+
CB10	0.222	AMP ERY	Weak	+	–	–	+	+	+
CB11	0.333	AMP TCN ERY	Moderate	+	–	–	+	+	+
CB12	0.333	AMP GEN ERY	Strong	+	–	–	+	+	+
CB13	0.222	AMP TCN	Weak	+	–	+	+	+	+
CB14	0.444	AMP CIP TCN ERY	Strong	+	+	–	+	+	+
CB15	0.222	AMP ERY	Weak	+	–	–	+	+	+
CB16	0.333	AMP TCN ERY	Moderate	+	–	+	+	+	+
CB17	0.222	AMP ERY	Negative	+	–	–	–	–	–
CB18	0.222	TCN ERY	Weak	+	–	–	–	+	–
CB19	0.222	AMP ERY	Weak	+	–	+	+	+	+
CB20	0.444	AMP GEN TCN ERY	Moderate	+	+	–	+	+	+
CB21	0.111	AMP	Negative	+	–	–	–	–	–
CB22	0.444	AMP GEN TCN ERY	Weak	+	–	–	+	+	+
CB23	0.111	TCN	Negative	+	–	–	–	–	–
CB24	0.556	AMP CIP KAN TCN ERY	Strong	+	–	+	+	+	+
CB25	0.444	AMP GEN TCN ERY	Moderate	+	–	+	+	+	+
CB26	0.333	AMP TCN ERY	Weak	+	–	–	+	+	+
CB27	0.333	AMP CIP TCN	Moderate	+	–	–	+	+	+
CB28	0.444	AMP CIP TCN ERY	Moderate	+	–	+	+	+	+
CB29	0.333	AMP KAN ERY	Moderate	+	–	–	+	+	+
CB30	0.222	KAN ERY	Weak	+	–	–	+	+	+
CB31	0.333	AMP TCN ERY	Weak	+	+	–	+	+	+
CB32	0.444	AMP CIP TCN ERY	Moderate	+	–	–	+	+	+
CB33	0.333	AMP TCN ERY	Weak	+	–	–	–	–	–
CB34	0.333	AMP CIP ERY	Weak	+	–	–	–	–	–
CB35	0.333	AMP TCN ERY	Weak	+	-	-	+	+	+
CB36	0.444	AMP CIP GEN TCN	Strong	+	-	+	+	+	+
CB37	0.333	AMP TCN ERY	Negative	+	-	-	-	-	-

AMP, ampicillin; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; TCN, tetracycline; ERY, erythromycin; +, positive; –, negative.

Table 3. Antimicrobial resistance pattern of *Vibrio parahaemolyticus* isolates

Antimicrobial agents	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	5 (13.5)	0 (0)	32 (86.5)
Ciprofloxacin	24 (64.9)	3 (8.1)	10 (27.0)
Kanamycin	27 (73.0)	5 (13.5)	5 (13.5)
Gentamicin	28 (75.7)	3 (8.1)	6 (16.2)
Tetracycline	7 (18.9)	3 (8.1)	27 (73.0)
Chloramphenicol	34 (91.9)	3 (8.1)	0 (0)
Erythromycin	3 (8.1)	6 (16.2)	28 (75.7)
Ceftazidime	37 (100.0)	0 (0)	0 (0)
Cefotaxime	35 (94.6)	2 (5.4)	0 (0)

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gentamicin (10.8%, 4/37). In contrast, a majority of the isolates were resistant to ampicillin (86.5%, 32/37), erythromycin (75.7%, 28/37), and tetracycline (73.0%, 27/37).

The multidrug resistance profile of 37 *V. parahaemolyticus* strains was also examined (Table 2). All 37 *V. parahaemolyticus* isolates were found to be resistant to at least 1 antibiotic, among which 2 (5.4%), 10 (27.0%), 16 (43.2%), 7 (18.9%), and 2 (5.4%) isolates were resistant to 1, 2, 3, 4, and 5 antibiotics, respectively. Overall, the MAR index ranged from 0.11 to 0.55 (Table 2), with 18 different resistance patterns. The highest frequencies of multidrug-resistant phenotypes were observed for ampicillin, tetracycline, and erythromycin (AMP TCN ERY) with 24.32%. Additionally, 10.81% and 8.1% of isolates exhibited the (AMP TCN) and (AMP GEN TCN ERY) as well as (AMP CIP TCN ERY) phenotypes, respectively.

ARGs in *V. parahaemolyticus* Isolates

The prevalence of 8 ARGs in *V. parahaemolyticus* isolates was examined. As shown in Table 4, *aac(3)-IV*, *qnrA*, and *tetA* were detected in 16.2%, 27.0%, and 62.2% of isolates, whereas blaTEM, blaOXA, blaSHV, ermA, and ermB were undetected in all isolates tested. Six isolates were *aac(3)-IV*-positive, 4 were susceptible to gentamicin, and 2 had intermediate resistance to gentamicin. All 10 *qnrA*-positive isolates were resistant to ciprofloxacin. Of 27 isolates exhibiting resistance to tetracycline, 23 isolates harbored the *tetA* gene.

Biofilm Formation of *V. parahaemolyticus* Isolates

The ability of *V. parahaemolyticus* isolates to form biofilms was investigated, as depicted in Figure 2. Out of the isolates tested, 31 (83.8%) were capable of producing biofilms, whereas 6 (16.2%) could not form biofilms. Within the group of 25 multidrug-resistant isolates, 4 were identified as strong biofilm producers, adhering robustly to polystyrene. Additionally, 11 isolates were classified as moderate biofilm producers, 9 as weak biofilm producers, and only 1 isolate (VA37) was unable to produce biofilm. Conversely, among the 12 non-multidrug-resistant isolates, a single isolate demonstrated moderate biofilm formation, 6 were weak biofilm producers, and 5 were incapable of forming biofilms.

Discussion

V. parahaemolyticus is an opportunistic pathogen and a major causative agent of food-borne illnesses worldwide [7]. This bacterium causes gastroenteritis following the consumption of contaminated raw or inadequately cooked seafood. Additionally, exposure to water containing *V. parahaemolyticus* can lead to wound infections and septicemia, which can be life-threatening for individuals with certain medical conditions, such as diabetes or immune deficiency [7]. The wide distribution of *V. parahaemolyticus* in tropical seawater and mariculture environments in different countries has been reported [20–23] and its presence depends on the water temperature [13]. In this study, *V. parahaemolyticus* was present in 24.7% (37/150) of samples, and it was more frequently identified in summer (35.1%) than in winter (14.5%). Previous studies have also noted a higher prevalence of *V. parahaemolyticus* in summer and autumn than in winter and spring [13,24]. This finding supports the hypothesis that the presence of *V. parahaemolyticus* is associated with a seasonal pattern, with outbreaks in the warmer season and lower prevalence in the cold season [25]. Since *V. parahaemolyticus* is cold-susceptible, its growth may be inhibited at lower temperature in winter [7], while higher temperatures in summer promote *V. parahaemolyticus* growth with a shorter generation time and a faster growth rate, leading to a higher density of *V. parahaemolyticus* in samples [26].

It is well known that not all *V. parahaemolyticus* strains cause diseases in aquatic animals and humans, but strains harboring the *tdh* and *trh* genes produce hemolysin factors that induce inflammatory gastroenteritis, and these genes are considered as virulent indicators of pathogenic

Table 4. The presence of antibiotic resistance genes in *Vibrio parahaemolyticus* isolates and the correlation between genotype and phenotype

Antimicrobial agent	Resistance genes	No. of positive isolates (%)	Correlation between genotype and phenotype
Ampicillin (*n* = 37)	bla_{TEM} bla_{OXA} bla_{SHV}	0 (0)	0 (0)
Gentamicin (*n* = 37)	*aac(3)-IV*	6 (16.2)	0 (0)
Ciprofloxacin (*n* = 37)	*qnrA*	10 (27.0)	10/10
Tetracycline (*n* = 37)	*tetA*	23 (62.2)	23/27
Erythromycin (*n* = 37)	*ermB* *ermA*	0 (0)	0 (0)

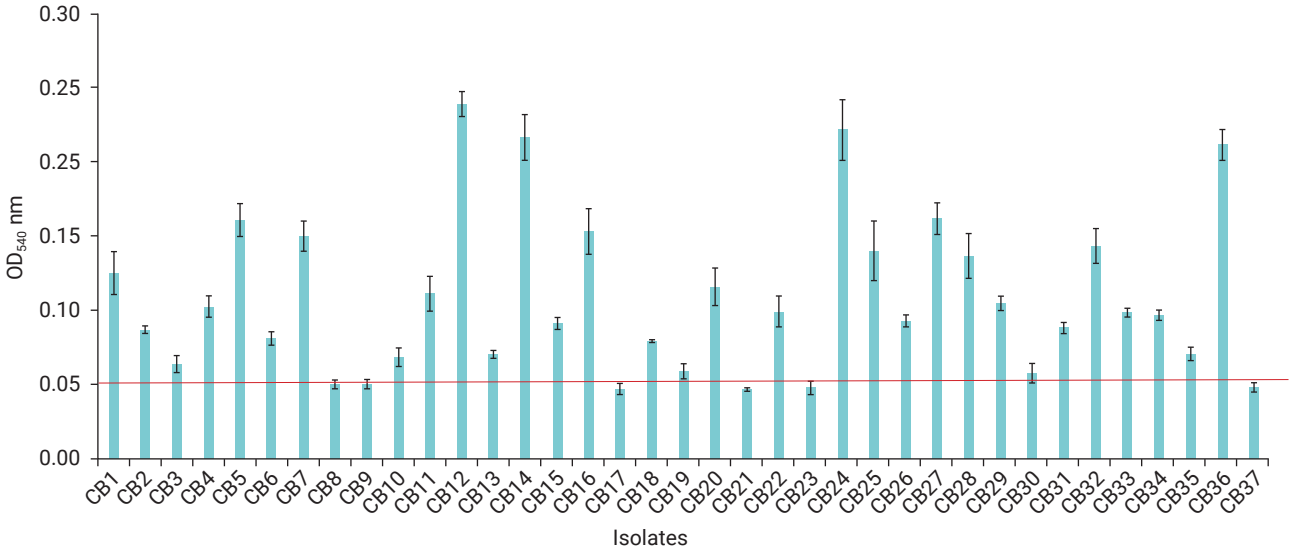

Figure 2. Biofilm formation of *Vibrio parahaemolyticus* isolates. The capacity of isolates to produce biofilms was interpreted via optical density $(OD)_{540}$ measurement. Error bars represent standard deviation. The red line shows control measurements.

strains [5]. Therefore, detecting these genes in isolates is crucial for mitigating potential risks to human health. In this study, we identified a significantly higher prevalence of the *trh* gene (21.6%) than the *tdh* gene (8.1%) among 37 *V. parahaemolyticus* isolates. This pattern aligns with previous research, which found the *trh* and *tdh* genes in 15.9% and 6.1% of seawater isolates from Korea [25] and in 19.8% and 9.9% of isolates from seafood in China [27], respectively. In contrast, the *tdh* gene was detected at a higher level (48%) than the *trh* gene (8.3%) in an estuarine system in South Carolina, the United States in a study by Gutierrez et al. [28] in 2013. Similarly, *tdh*-positive *V. parahaemolyticus* was also more prevalent than *trh*-positive bacteria in oyster environments in Taiwan [29] and in coastal water in Saudi Arabia [30]. The differences in the distribution of tdh^+/trh^+ *V. parahaemolyticus* strains are possibly due to the sampling techniques, the geographical origin, seasonal effects and the method of their detection [12]. For example, Parveen et al. [31] reported that real-time PCR could help increase the detection of *tdh*- and *trh*-positive *V. parahaemolyticus* to 13% and 40%, respectively, for water samples compared to the conventional techniques.

The antimicrobial resistance of *Vibrio* species has emerged as one of the most significant threats to fish farming, food safety, and public health [32]. Therefore, monitoring the antimicrobial susceptibility of *V. parahaemolyticus* is very important for evaluating its potential effects on environmental and human health. Our results indicated that more than 72% of *V. parahaemolyticus* isolates from mariculture were resistant to ampicillin, erythromycin, and tetracycline. These findings broadly support the work of other studies, in which 86% to 100% of *V. parahaemolyticus* strains isolated from marine environments [33,34] were resistant to ampicillin and tetracycline, and 42% to 48.3% of isolates from shrimp mariculture were resistant to erythromycin [12,35]. This high frequency of resistance confirms that these antimicrobials are widely used and becoming less effective against *V. parahaemolyticus*, likely due to the extensive use of antibiotics in the areas studied. In Vietnam, 64% of fish farms reported using at least 1 antibiotic for disease treatment and prevention, with 10% to 21% of farms utilizing tetracycline [36]. Additionally, significant levels of antibiotic residues, including ampicillin, erythromycin, and tetracycline, have been detected in aquaculture water in Vietnam, which may contribute to the increasing resistance rates to these antimicrobials [37]. Conversely, other studies have shown that ampicillin, tetracycline, and erythromycin were effective against *V. parahaemolyticus* strains, with over 90% of isolates being inhibited [10,38–40]. The variability in resistance of *Vibrio* to antibiotics may be attributed to geographical differences or variations in testing methodologies [5].

Significantly, all tested *V. parahaemolyticus* isolates were found to be susceptible to third-generation cephalosporins (ceftazidime and cefotaxime) and chloramphenicol. However, 2 isolates showed intermediate resistance to cefotaxime, and 3 isolates exhibited intermediate resistance to chloramphenicol. These findings are consistent with those of previous studies [12,13,39,41], which reported that over 90% of *V. parahaemolyticus* strains were susceptible to these antibiotics. Therefore, these drugs are considered the most

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effective antimicrobials for treating *V. parahaemolyticus* infections in the areas studied.

Ciprofloxacin, a fluoroquinolone antibiotic, has been broadly employed as an alternative treatment for tetracycline-resistant bacteria [33], while aminoglycosides (kanamycin and gentamicin) antibiotics are commonly used in aquaculture production and show substantial effectiveness against a broad spectrum of bacteria [42]. However, the use of these antibiotics for the prevention and treatment of *V. parahaemolyticus* may not be effective, as the rate of antibiotic resistance among 37 *V. parahaemolyticus* isolates in the current study ranged from 13.5% to 27.0%. Comparable resistance rates have been observed in previous studies, with 9.5% to 39.8% of *V. parahaemolyticus* strains showing resistance to kanamycin, ciprofloxacin, and gentamicin [24,43]. The increasing resistance of *V. parahaemolyticus* to fluoroquinolones and aminoglycosides could be attributed to their extensive use in human medicine and aquaculture production, which may lead to significant public health concerns [44].

MAR indexing has been recognized as an efficient and cost-effective method for tracking the sources of bacterial contamination. Letchumanan et al. [5] suggest that MAR index values greater than 0.2 indicate high-risk sources of antibiotic contamination, posing a potential threat to human health. Regrettably, our study revealed that 67.57% of *V. parahaemolyticus* isolates had a MAR index exceeding 0.2, indicating a multidrug resistance phenotype. Our findings suggest that these *V. parahaemolyticus* strains were recovered from sources where antibiotics are frequently used, which could be hazardous. This is in contrast to the findings of Mok et al. [13], who reported that only 1.9% of *V. parahaemolyticus* strains isolated from the Korean coast were resistant to 3 antibiotics. Nonetheless, our results are in general agreement with previous observations [6,24,45], which showed that more than half of the environmental *V. parahaemolyticus* isolates exhibited multidrug resistance. Furthermore, the MAR index values in this study ranged from 0.11 to 0.55, which are significantly lower than those reported by Ahmed et al. [46], who found all *V. parahaemolyticus* isolates were resistant to at least 7 antimicrobial agents. The variation in the MAR index values may be attributed to differences in sample sources, geographic distribution, the number and types of antibiotics tested, and the methodologies employed [47].

The presence of ARGs is the basis for bacterial resistance, and a high detection rate of ARGs indicates an elevated risk of ARG transmission and a significant potential for bacteria to develop resistance [48]. In the present study, the *qnrA* gene was detected in all 10 ciprofloxacin-resistant isolates, demonstrating a strong correlation between phenotypic resistance and genotype (the presence of *qnrA*). This result aligns with previous research [44,45], in which all fluoroquinolone-resistant isolates carried the *qnrA* and *qnrS* genes. Conversely, Jeamsripong et al. [49] showed that 77.8% of 594 *V. parahaemolyticus* isolates harbored the *qnr* gene, but all isolates were susceptible to ciprofloxacin. This difference is possibly due to the evolution, mutation, and silencing of resistance genes [50] or unknown mechanisms that remain to be elucidated.

Similarly, a strong relationship between the tetracycline resistance phenotypes of *V. parahaemolyticus* isolates and the presence of ARG was also determined, as *tetA* was detected in 23 of 27 isolates that exhibited resistance to tetracycline. The overall level was found to be much higher than reported in other studies [45,51] in which the detection rate of *tetA* was 28% to 30% of tetracycline-resistant isolates. Meanwhile, *tetA* was undetected in all *V. parahaemolyticus* strains that exhibited resistance to tetracycline [5,52]. A possible explanation for this difference might be that *V. parahaemolyticus* strains used other ARGs (*tetM* and *tetS*) against tetracycline [53].

Interestingly, a high percentage of isolates showed resistance to ampicillin and/or erythromycin; however, none of the 32 or 28 isolates resistant to ampicillin/erythromycin carried the genes for macrolide or β-lactam antibiotic resistance. This discrepancy between the resistance phenotypes and genotypes for ampicillin and/or erythromycin has been noted in previous studies [5,12,45]. This may occur because resistance phenotypes can be governed by various ARGs and mechanisms [12]. Another potential explanation is that the resistance phenotypes are regulated by the efflux systems of the bacterial cells [54].

The *aac(3)-IV* gene was detected in susceptible and intermediate-resistance isolates, but not in gentamicin-resistant strains. Similarly, Beshiru and Igbinosa [45] only found the *aac(3)-IV* gene in 2 intermediate gentamicin-resistant isolates. However, the findings of the current study do not support the previous research [12], in which positive results were found for the *aac(3)-IV* gene in 12 of 61 isolates that exhibited resistance to gentamicin. Nevertheless, susceptible isolates harbored the *aac(3)-IV* gene, posing a potential risk for the preservation and transmission of ARGs [12].

Biofilm formation is a significant virulence factor for pathogenic bacteria during infection. Several *Vibrio* species, including *V. parahaemolyticus*, can produce biofilms, enabling the bacteria to establish infections and enhance their resistance to hostile environments, such as antibiotics and the host immune response [55]. This study found that more than 83% of *V. parahaemolyticus* isolates were biofilm

producers (Figure 2). The rate observed in this investigation was 16% lower than that of previous studies, in which 100% of *V. parahaemolyticus* strains isolated from aquatic animals and marine environments [47,56,57] were able to produce biofilms. The difference in the capacity to form biofilm may be due to the physical conditions (temperature, pH, etc.), and the surfaces where cells attach [58]. Another important finding is that isolates with multidrug resistance (64.9%, 24/37) were more likely to produce biofilms than non-multidrug-resistant isolates (18.9%, 7/37). This supports the possibility that biofilm formation is associated with increased antimicrobial resistance, as suggested in the literature [59–61]. Moreover, the *trh* gene was detected in 6 biofilm-producing strains that were resistant to 4 or 5 antibiotics. This outcome is consistent with Letchumanan's findings [62], which indicated that 9 out of 13 *trh*-positive strains exhibited resistance to 4 or more antimicrobials. Interestingly, a correlation between biofilm phenotype and associated genes was also identified in this study, as 3 genes (*VP0950*, *VP0952*, and *VP0962*) that mediate biofilm formation were present in *V. parahaemolyticus* isolates that were identified as biofilm producers. Similar results were also reported in other studies [47,56].

The paper presents intriguing findings on the prevalence of ARGs and the virulence genes of *V. parahaemolyticus* strains in aquaculture environments and their implications for public health. However, this study did not assess the prevalence of *V. parahaemolyticus* in fish, which could pose a direct threat to human health. Nevertheless, it is widely recognized that *V. parahaemolyticus* is an adept swimmer capable of attaching to aquatic animals [63]. Thus, a contaminated water body may potentially infect all its fish inhabitants [64]. Alarmingly, some *V. parahaemolyticus* isolates identified in the current study carry ARGs and virulence genes, which could pose a significant risk to human health. Aquatic ecosystems, particularly, are acknowledged as hotspots for the environmental spread of pathogenic microorganisms, ARGs, and antimicrobials [1]. Furthermore, the intricate environmental conditions of tropical aquatic habitats may prompt genetic differentiation and adaptive variation in organisms. These changes can enhance their pathogenicity or lead to the emergence of new strains with distinct virulence factors in aquatic hosts [32]. Therefore, to better understand the genotypic variation of *V. parahaemolyticus* isolates, to determine the relatedness between clinical and environmental isolates, and to clarify the relationships of *V. parahaemolyticus* virulence genes across different marine environmental sources, further research is required.

Conclusion

This study provides the first insights into the prevalence, antimicrobial resistance profile, distribution of resistance genes, and virulence genes of *V. parahaemolyticus* strains isolated from fish mariculture environments in Vietnam. The findings suggest that rearing water is a potential source of antimicrobial-resistant *V. parahaemolyticus* strains, posing a risk to both aquatic animal and human health. The high frequency of resistance to several antibiotics, including ampicillin, erythromycin, and tetracycline, as well as the presence of multidrug-resistant isolates, points to the extensive use of these antibiotics in the region, which should be strictly controlled to prevent their potential spread to humans. Notably, the capacity of several multidrug-resistant isolates to produce strong biofilms indicates the persistence of *V. parahaemolyticus* strains in marine environments. Therefore, continuous monitoring of *V. parahaemolyticus* and ARGs in seafood and the mariculture environment is crucial to mitigate potential risks to human health.

Supplementary Material

Figure S1. Map of the sampling locations in Cat Ba, Hai Phong, Vietnam. The red dots indicate the places of sampling; **Table S1.** List of sampling locations and number of collected samples; **Table S2.** List of primers used for detection of *Vibrio parahaemolyticus*, antimicrobial resistance genes, and virulence genes. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0181.

Notes

Ethics Approval
Not applicable.

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

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Availability of Data
All data generated or analyzed during this study are included in this published article (and its Supplementary Information files). For other data, these may be requested through the corresponding author.

Authors' Contributions
Conceptualization: PVN, KCTN; Data curation: PHT, HTT, XTH; Formal analysis: KCTN; Funding acquisition: KCTN; Investigation: all authors; Writing–original draft: PVN, KCTN; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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Original Article

Epidemiological analysis and prevention strategies in response to a shigellosis cluster outbreak: a retrospective case series in an alternative school in the Republic of Korea, 2023

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ABSTRACT

Objectives: In March 2023, an alternative school in the Republic of Korea reported 12 cases of shigellosis. This study aims to analyze the epidemiological characteristics in order to determine the cause of the cluster outbreak of shigellosis and to develop prevention strategies.
Methods: This study focused on 12 patients with confirmed *Shigella* infection and investigated their demographics, clinical features, epidemiology, diagnostics, and antimicrobial susceptibility. Following the identification of *Shigella*, we conducted follow-up rectal smear cultures to manage patients, implementing isolation and control measures.
Results: This study investigated the emergence of multidrug-resistant *Shigella* following missionary activities in Cambodia, documenting a cluster infection within an alternative school in Daejeon, the Republic of Korea. The outbreak affected 56 participants, resulting in the confirmation of 12 cases. The incidence rates varied by gender and occupation, with higher rates among males and teachers. All 12 cases demonstrated multidrug resistance. Challenges included delayed pathogen confirmation and suboptimal adherence to isolation criteria. The incident prompted revisions in the criteria for isolation release, focusing on symptom resolution. The study underscores the necessity for strengthened surveillance, educational initiatives focusing on prevention in endemic areas, and improved oversight of unlicensed educational establishments.
Conclusion: Successful response strategies included swift situation assessment, collaborative efforts, effective infection control measures, and modified criteria for isolation release. Continued surveillance of multidrug-resistant strains is recommended, especially in regions with a high prevalence.

Keywords: Alternative; Disease outbreaks; Education; *Shigella sonnei*

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Introduction

Shigella species are important pathogens that cause diarrhea and intestinal infections in developing and developed countries [1,2]. The World Health Organization (WHO) estimates that *Shigella* is responsible for at least 80 million cases of bloody diarrhea and contributes to approximately 7 million deaths worldwide each year [3]. In 2016, despite significant reductions in mortality over the past 3 decades, shigellosis was associated with roughly 212,438 deaths across all age groups, including about 60,000 fatalities among children under 5 years of age [4]. In the United States, 52 state and regional public health laboratories reported 12,597 cases of culture-confirmed *Shigella* spp. to the Laboratory-based Enteric Disease Surveillance system in 2016 [5]. In the Republic of Korea, the number of reported cases was 151 in 2019, decreased to 29 in 2020, then to 18 in 2021, increased to 31 in 2022, and stood at 17 by April 2023 [6]. Shigellosis is diagnosed through the isolation and identification of *Shigella* bacteria from stool or rectal swab samples. The primary symptoms of shigellosis vary and can include high fever, nausea, vomiting, crampy abdominal pain, and diarrhea, which may contain blood or mucus. In some cases, the infection may be asymptomatic. The typical incubation period for shigellosis is up to 14 days [1]. It is important to note that the cornerstone of shigellosis treatment is the maintenance of hydration and electrolyte balance [7]. Furthermore, the infection remains contagious until the bacteria are no longer present in the stool, which usually occurs within a few days to 4 weeks after symptoms begin. In rare instances, individuals can carry the bacteria for several months [1]. Among the various *Shigella* spp., *Shigella sonnei* is the most commonly isolated species in shigellosis cases in industrialized countries [8]. Over recent decades, it has also become increasingly prevalent throughout Southeast Asia [9].

The rise of multidrug-resistant (MDR) strains within *Enterobacteriaceae*, notably *Shigella*, presents a significant threat to public health. This resistance compromises the efficacy of treatments, leading to longer durations of illness, increased healthcare costs, and higher mortality rates. Recent studies have highlighted the link between drug resistance and the lack of clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) systems in *Enterobacteriaceae*, especially in the context of clinical infections [10,11]. In a tertiary hospital in the Republic of Korea, research focused on carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* (CRE/CRAB) [12]. Although community-acquired cases were relatively rare (6%), the study found that 20% of CRE/CRAB isolates were already present. The detection of carbapenem-resistant bacteria in domestic pets and their environments highlights the need to include these animals in One Health surveillance efforts to more effectively combat antimicrobial resistance [13]. Data on the response to overseas-imported shigellosis in alternative schools are limited. Therefore, the purpose of this study is to provide an epidemiological analysis of patients with overseas-imported shigellosis in an alternative school and to document their response status. The goal is to lay the groundwork for developing strategies to prevent the spread of shigellosis in future incidents.

HIGHLIGHTS

- In March 2023, a shigellosis outbreak affected 12 individuals at an alternative school in the Republic of Korea.
- This study aimed to analyze and prevent cluster outbreaks.
- *Shigella* cases were confirmed in 21.4% of individuals engaged in missionary activities in Cambodia, all of whom presented with diarrhea.
- The strains exhibited multidrug resistance.
- Rapid identification, on-site investigations, and strict hygiene measures halted the epidemic without further local transmission.
- Swift situational awareness and responses are crucial for interrupting transmission and preventing the spread of *Shigella*.
- Collective efforts from schools, families, and communities can improve patient compliance with infection control measures.

Materials and Methods

Study Background and Settings

On March 28, 2023, at 16:00, a medical institution in Daejeon, the Republic of Korea, reported a case of shigellosis, which was then recorded in the integrated disease control and prevention management system. Upon verification of the report, the public health office identified a student who had begun showing symptoms, including fever and diarrhea, on March 25, 2023. The student was subsequently subjected to a rectal swab test. Results from the test, conducted on March 28, 2023, detected the *Shigella*-specific gene (ipaH), confirming the diagnosis of *Shigella* infection in this individual.

An epidemiological study was initiated by a local public health office. The investigation revealed that a student had engaged in mission work in Cambodia for 6 days, from March 20 to March 25, 2023. The student reported that several friends who also participated in the missionary activities exhibited symptoms such as fever and diarrhea. Notably, a friend who shared a room with the student had earlier experienced diarrhea and a high fever. In response to this cluster of symptomatic individuals suggestive of shigellosis, the local health department reported the situation to Daejeon and conducted an on-site epidemiological investigation on March 29, in collaboration with epidemiological investigation personnel from Daejeon City.

The epidemiological investigation was coordinated by the Daejeon Metropolitan City Epidemiological Investigation Team and supported by the Chungcheong Reginal Center for Disease Control and Prevention. This collaboration also involved the local public health offices (4 in total), which were selected based on the patient's area of residence.

The school under investigation was unlicensed and affiliated with an alternative religion, serving approximately 600 students and 100 staff members. It offered educational programs for elementary, middle, and high school levels. The middle school curriculum incorporated overseas missionary work. In March 2023, a group of 51 third-grade middle school students and 5 teachers from the school participated in a mission trip to Siem Reap, Cambodia, from March 20 to March 25. Following this trip, 12 individuals—10 students from the 3rd grade of middle school and 2 teachers—contracted *Shigella*.

Epidemiological Investigation

Case definition

Individuals who engaged in missionary activities in Cambodia from March 20 to March 25, 2023, and subsequently displayed clinical symptoms indicative of shigellosis, with the presence of *Shigella* bacteria confirmed through the isolation and identification from their stool or rectal swab samples, were defined as cases.

Study design

We attempted to estimate the source of infection through a cohort or case-control study. However, we faced limitations related to assessing food consumption during missionary activities in Cambodia due to the lack of dietary records, such as those for hotel breakfasts and group meals. Consequently, we opted to conduct a case series study of individuals who met the case definition.

Case Investigation

Patient information was collected through face-to-face interviews or telephone conversations using a shigellosis Epidemiological Investigation Form. The collected information included general characteristics, such as sex, age, place of residence, and occupation, and diagnostic and reporting-related data, such as the reporting classification and type of pathogen. The clinical symptom information collected included the date and time of initial symptom onset, symptoms and signs, medical facility visits, duration of illness, antibiotic treatment, and underlying diseases. Epidemiological data were obtained by investigating food consumption, international travel histories, and participation in group meals.

Laboratory Testing

Rectal swab culture tests were conducted for 16 types of bacteria, 5 types of viruses, and 4 types of parasites at the National Institute of Health and Environment. Students and school staff underwent rectal swab tests at public health centers near the school. Family members living in the same household were instructed to visit their local public health centers for the same tests. The samples were then sent to the local National Institute of Health and Environment for analysis. Additionally, *Shigella* isolates underwent confirmation, genotyping, and antimicrobial susceptibility testing at the Korea Disease Control and Prevention Agency (KDCA) to determine genetic relatedness among the pathogens. The identification of *Shigella* spp. isolates was performed using the VITEK 2 system (BioMerieux), and serotyping was carried out with commercial antisera kits (Denka Seiken) designed for all *Shigella* spp. serovars. Antimicrobial susceptibility testing for these isolates was conducted using the broth microdilution method with the Sensititre KRCDC2F custom panel (TREK Diagnostic Systems), following the Clinical and Laboratory Standards Institute guidelines. All isolates were analyzed by pulsed-field gel electrophoresis (PFGE) after *XbaI* digestion, in compliance with the PulseNet International protocol (https://pulsenetinternational.org/). The genetic relatedness among the PFGE patterns was assessed using BioNumerics ver. 7.6 (Applied Maths).

Follow-Up Investigation

Twelve patients diagnosed with shigellosis underwent both inpatient and outpatient treatments. Upon completion of their treatment, they were subjected to follow-up tests using rectal swab cultures at 24-hour intervals to confirm the absence of the bacteria. Furthermore, individuals in close contact with the patients, particularly family members residing in the same household, were identified as close contacts and also underwent rectal swab testing.

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Data Analysis

Descriptive statistics, including frequency and percentage, were used to analyze the demographic and clinical characteristics of the cases gathered during the epidemiological investigation. All analyses were conducted using Microsoft Excel ver. 2013 (Microsoft Corp.)

Ethics Approval

The study protocol was approved by the Institutional Review Board of the KDCA (No: KDCA-2023-09-04-PE-01).

Results

On March 28, 2023, a healthcare facility reported a suspected case of shigellosis to the local health department. The following day, officials from the local health department and regional authorities carried out an epidemiological investigation at the site. This investigation uncovered that a group of 56 individuals, comprising 51 students and 5 teachers, had participated in missionary activities in Cambodia from March 20 to March 25, 2023. Beyond the initial reported case, multiple individuals exhibiting symptoms were also identified.

In response to these findings, stool culture tests were administered on March 30 and 31, 2023, to 55 individuals who had participated in the missionary activities on both days, with the exception of the first case who had already tested positive via polymerase chain reaction (PCR). These individuals were also subjected to workplace swab tests. The National Institute of Environmental Research later reported that, of the 55 individuals tested, 11 were found to be positive for *Shigella* on March 31, 2023. On the same day, the local health department recommended that those who tested positive via PCR should self-isolate and highlighted the critical importance of maintaining personal hygiene. In addition, *Shigella* culture tests were performed on the close family members of the PCR-positive individuals, but only for those who had given verbal consent.

Of the 12 PCR-positive individuals reported on March 31, 2023, the National Institute of Environmental Research confirmed that *S. sonnei* was cultured from 6 of them on April 4, 2023, and another 6 on April 7, 2023. These 12 individuals fell under the jurisdiction of 4 local health departments. Each department was notified of the positive *S. sonnei* culture results to effectively manage the cases. They were provided with guidance on patient management, which included isolating confirmed cases and conducting additional testing before releasing them from isolation. Of the 27 family members of patients with *Shigella*, 12 consented to participate in *Shigella* testing, all of whom tested negative. The remaining 15 individuals chose not to undergo testing; however, they were informed about the importance of personal hygiene.

Of the 56 individuals in the study, who comprised 51 students and 5 teachers from an alternative school who participated in missionary activities in Cambodia over a period of 6 days from March 20 to March 25, 2023, 12 tested positive for *Shigella* on culture examinations. When examining demographic characteristics, the incidence rate was higher in males (25.8%; 8 out of 31) than in females (16.0%; 4 out of 25). Furthermore, the incidence rate among teachers was significantly higher (40.0%; 2 out of 5) than among students (19.6%; 10 out of 51) (Table 1).

The epidemiological analysis of shigellosis cases indicated that the onset of symptoms occurred on March 22, 23, 24, and 25, 2023, affecting 3, 3, 2, and 4 individuals, respectively. Taking into account the known incubation period for *Shigella*, which ranges from 12 hours to 7 days, we determined that the likely exposure period corresponded with missionary activities in Cambodia, as shown in Figure 1. Students and teachers participated in missionary activities in Siem Reap, Cambodia, from March 20 to 25, 2023. Individual bottled

Table 1. *Shigella sonnei* attack rate by age, gender, and identity among the participants in overseas missionary activities ($n = 56$)

Variable	Total (n)	*Shigella sonnei* case (n)	Attack rate (%)
Total	56	12	21.4
Sex			
Male	31	8	25.8
Female	25	4	16.0
Identity			
Teacher	5	2	40.0
Student	51	10	19.6

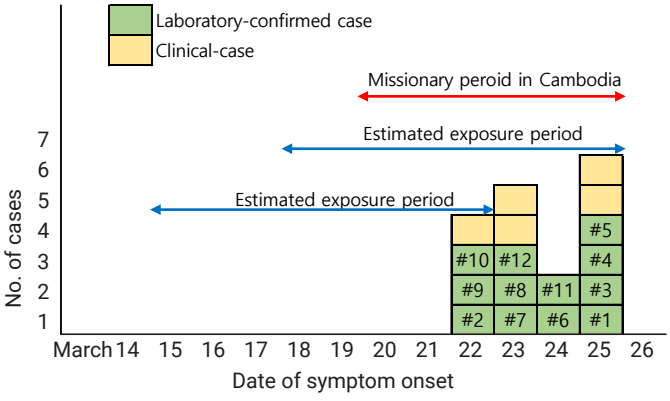

Figure 1. Number of laboratory laboratory-confirmed and clinical *Shigella sonnei* cases.

containers were provided for drinking water. The hotel's menus, as reported by school officials, included kimchi stew, braised ribs, pork, beef, miso, army stew, morning glory stir-fry, and a variety of tropical fruits such as mango, longan, watermelon, papaya, and pineapple. However, the accuracy of an individual's dietary information is dependent on memory and cannot be precisely determined. All 12 individuals who tested positive for *Shigella* in the culture examination experienced symptoms of diarrhea, with the number of episodes ranging from 3 to 10. The most common accompanying symptoms were abdominal pain, reported in 9 cases, fever in 6, and headaches in 5 (Table 2).

In the patient isolate analysis, 2 PFGE patterns were detected using the *XbaI* restriction enzyme. These patterns were identified as SZNX01.183, which displayed 11 bands, and SZNX01.300, which consisted of a single band. The genetic relatedness between these 2 patterns was found to be 97.98%. Antibiotic susceptibility testing indicated that the *Shigella* strains were resistant to multiple antibiotics, including ampicillin, and exhibited a MDR phenotype, showing resistance to 7 or 8 different antibiotics (Table 2). According to the PulseNet database for domestic *S. sonnei*, the SZNX01.183 pattern with 11 bands has been previously identified in *Shigella* strains that were introduced from foreign countries, including India, Cambodia, and Vietnam, between the years 2012 and 2014 (Figure 2).

The epidemiological investigation conducted in this study successfully isolated *Shigella* from the stool samples of 12 individuals. Serogroup D of *S. sonnei* was identified and confirmed as the causative pathogen. Although the duration of the individuals' stay in Cambodia coincided with the incubation period of *Shigella* (Figure 1), the lack of sufficient evidence from food consumption and environmental investigations precluded the determination of the precise source of infection.

Shigella-positive individuals were monitored, and subsequent second and third culture tests were conducted to determine when they could be released from isolation. Following the confirmation of negative results, there were no further cases of shigellosis reported as of May 24, 2023, marking the end of the outbreak (Figure 3).

Discussion

Shigellosis, also known as bacillary dysentery, is officially recognized as a notifiable infectious disease under the Communicable Disease Prevention Act enforced by the KDCA. It falls under the management framework of Class 2 infectious diseases according to legal regulations. The KDCA reports that the incidence of shigellosis in the Republic of Korea has decreased since the 1950s, following the introduction of antibiotics and advancements in environmental hygiene. During the global coronavirus disease 2019 (COVID-19) pandemic, from 2020 to 2022, there was a notable decline in cases of shigellosis acquired overseas in the Republic of Korea. The recorded cases for these years were 29 in 2020, 18 in 2021, and 31 in 2022 [14]. This trend aligns with the observed decrease in gastrointestinal infections during the COVID-19 pandemic in the United Kingdom [15].

Shigellosis can cause a wide range of clinical symptoms, ranging from asymptomatic cases to mild or severe illness, which may include dehydration and bloody diarrhea [1]. In the outbreak described, all patients exhibited the typical symptom of watery diarrhea, while other symptoms such as fever, nausea, and vomiting differed among individuals. These observations align with the symptomatology reported in prior research on shigellosis [16].

All 12 cases of *S. sonnei* identified in this study were found to be MDR strains, showing resistance to 7 or 8 different antibiotics based on antibiotic susceptibility analysis. *S. sonnei* is globally distributed and is the most common causative agent of shigellosis in industrialized regions, such as Europe, North America, and Australia. It is currently expanding to middle-income countries across Asia, Latin America, and the Middle East [17]. In acute pediatric diarrheal samples collected in Tehran, *S. sonnei* was detected in 40 out of 75 *Shigella* spp. cases [18].

S. sonnei has demonstrated MDR to sulfonamides, ampicillin, streptomycin, and tetracycline since the 1960s, which suggests the presence of a variety of antimicrobial resistance genes [1,2]. As a result, the use of fluoroquinolones, especially ciprofloxacin, increased for treating drug-resistant shigellosis, and their use became the standard practice after the WHO endorsed them in 2005. However, the extensive use of fluoroquinolones, including ciprofloxacin, has led to the emergence of antibiotic resistance. By the late 1990s, ciprofloxacin-resistant *S. sonnei* had appeared and begun to spread across Asia [19–22]. For instance, between 2014 and 2019, 98% of *Shigella* isolates collected in Cambodia were found to be MDR [23].

Based on results from the PulseNet database of the KDCA, the *S. sonnei* strain implicated in the recent outbreak, identified as SZNX01.183, was found to match the type associated with overseas shigellosis cases imported from India, Cambodia, and Vietnam between 2012 and 2014. Consequently, it can be inferred that the shigellosis outbreak in this instance was linked to exposure incurred during missionary activities in Cambodia.

In this outbreak, it took some time to confirm that the gastrointestinal infection with symptoms of diarrhea was

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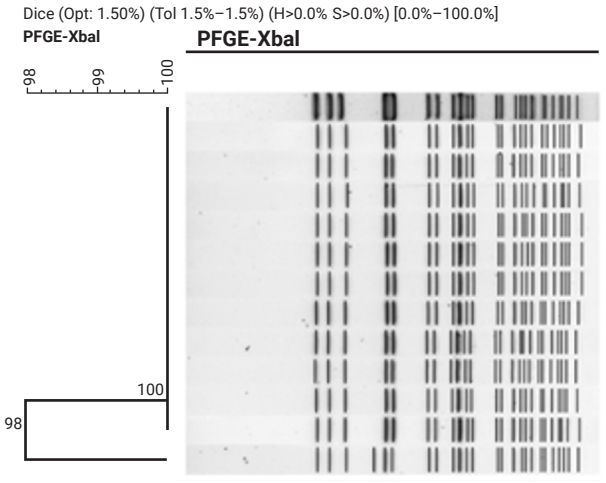
Table 2. Demographic and clinical characteristics of the *Shigella sonnei* patients ($n = 12$)

Characteristic	Case no.											
	1	2	3	4	5	6	7	8	9	10	11	12
Sex	Female	Male	Female	Male	Male	Male	Female	Male	Female	Male	Male	Male
Occupation	Teacher	Teacher	Student	Student	Student	Student	Student	Student	Student	Student	Student	Student
Symptoms												
Fever	N	N	Y	Y	N	Y	N	N	Y	Y	Y	N
Nausea	Y	N	N	Y	N	N	N	N	N	N	Y	N
Vomiting	Y	N	N	Y	N	N	N	N	Y	N	Y	N
Watery diarrhea[a)]	Y6	Y5	Y10	Y8	Y4	Y8	Y5	Y8	Y6	Y5	Y4	Y3
Muscle pain	Y	N	Y	Y	N	N	N	N	N	N	N	N
Headache	Y	N	N	N	Y	N	Y	Y	N	N	N	N
Stomachache	Y	Y	Y	N	N	N	N	Y	Y	Y	Y	Y
Dehydration	Y	N	N	N	N	N	N	N	N	N	N	N
Types of bacteria	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*
Serotype	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II	D, phase II
PFGE type	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.183	SZNX01.300	SZNX01.183
Antimicrobial susceptibility												
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R
Azithromycin	R	R	R	R	R	R	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R	R	R	R	R	R	R
Ceftriaxone	R	R	R	R	R	R	R	R	R	R	R	R
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R
Nalidixic acid	R	R	R	R	R	R	R	R	R	R	R	R
Tetracycline	R	R	R	S	R	S	R	R	R	R	S	R
Trimethoprim/ sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R

N, no; Y, yes; PFGE, pulsed-field gel electrophoresis; R, resistant; S, susceptible.

[a)]The no. represents the frequency of diarrhea episodes.



ID	Category	Years	PFGE patterns
A	Cambodia	2013	SZN01.183
3	Missionary activity	2023	SZN01.183
6	Missionary activity	2023	SZN01.183
8	Missionary activity	2023	SZN01.183
10	Missionary activity	2023	SZN01.183
4	Missionary activity	2023	SZN01.183
12	Missionary activity	2023	SZN01.183
7	Missionary activity	2023	SZN01.183
5	Missionary activity	2023	SZN01.183
9	Missionary activity	2023	SZN01.183
1	Missionary activity	2023	SZN01.183
2	Missionary activity	2023	SZN01.183
11	Missionary activity	2023	SZN01.300

Figure 2. *XbaI* pulsed-field gel electrophoresis (PFGE) patterns of *Shigella sonnei* strains identified during the 2023 outbreak in the Republic of Korea, as well as a strain isolated from individuals in Vietnam. Strain A originated in India, Cambodia, Vietnam. The scale bar indicates percentage relatedness.

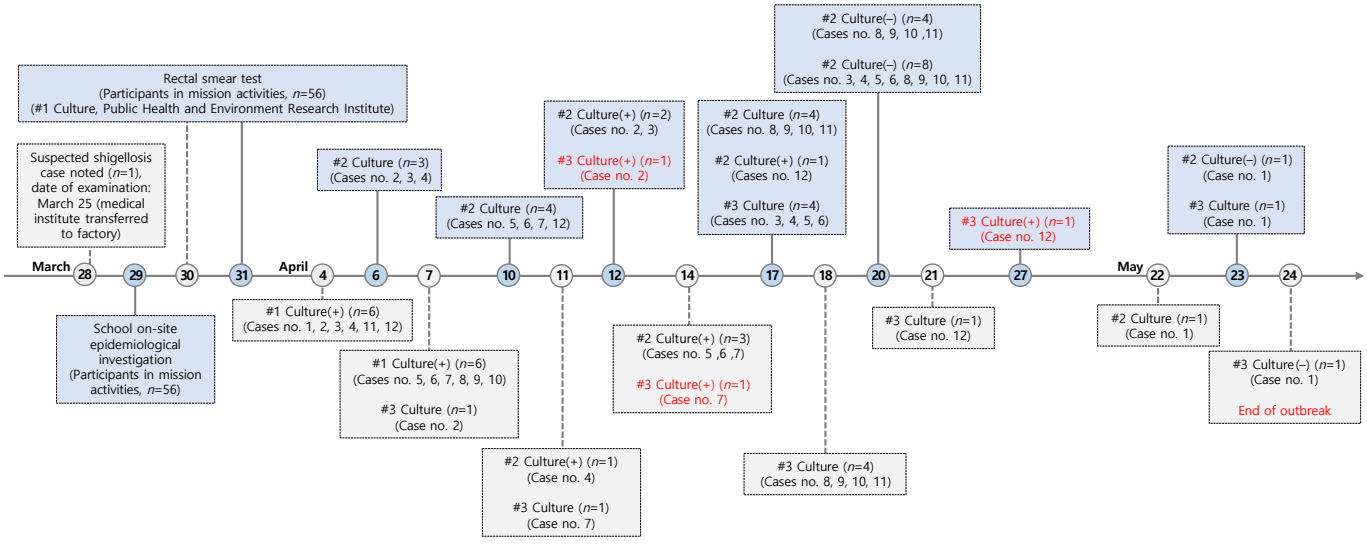


Figure 3. Timeline of the *Shigella sonnei* outbreak, from the initial recognition of the outbreak, through the on-site epidemiological investigation, to the conclusion of the outbreak in the school.

caused by MDR *Shigella*. Consequently, individuals who sought medical care before the test results were available received appropriate treatment. This could include antibiotics or symptomatic treatment, depending on the severity of their symptoms, in accordance with the travelers' diarrhea treatment guidelines [24]. However, a significant amount of time was required for individuals to meet the isolation release criteria, which necessitated 2 negative culture test results without the use of antibiotics. Despite the resolution of symptoms, some teachers remained unable to return to work, and students were not able to fully participate in classes, leading to less than optimal adherence to infection management. This outbreak prompted a reassessment of the need to reconsider the shigellosis isolation release criteria in the Republic of Korea. As a result, the KDCA revised the isolation release criteria to consider both the presence or absence of symptoms and laboratory test results.

The school involved in the recent shigellosis outbreak operated as an alternative educational institution. It was not subject to the School Health Act, functioning as an unlicensed educational facility outside the regulatory oversight of both the Ministry of Education and the provincial

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Office of Education. This situation has brought to light the problem of ambiguous authority over the management and supervision of such institutions. In a similar case from January 2021, an unlicensed residential educational facility run by a missionary group became the center of a COVID-19 cluster infection [25,26]. This incident further underscored the need for better regulation of unlicensed educational organizations. Previous research suggests that a practical approach might include the classification and mandatory reporting of various types of unlicensed educational facilities to protect students. Additionally, creating legal frameworks to govern and administer these reported institutions could be an effective solution [27].

The implementation of immediate school restrictions, self-isolation, and infection control measures, such as hand hygiene, upon confirmation of test results during the shigellosis outbreak, was effective in preventing further spread. These measures are consistent with non-pharmaceutical interventions that have been previously employed in the management of patients with shigellosis [28].

Prior research has demonstrated a widespread prevalence of MDR *Shigella* strains in Southeast Asia, underscoring the need for improved surveillance to control the spread of antibiotic resistance. This is particularly important given the potential for overseas travel to contribute to this issue [16,29].

Recent updates have been made to the criteria for releasing shigellosis patients from isolation, which are now based on the resolution of symptoms. Further research is needed to evaluate the awareness and acceptance of these revised guidelines among infection control personnel.

Conclusion

The successful response to the outbreak of MDR *Shigella* imported from Cambodia was facilitated by a rapid assessment of the situation and cooperative efforts between local communities and pertinent agencies. It is crucial to maintain timely communication with these agencies to prevent the spread of infection domestically after it is introduced from abroad. Furthermore, it is important to focus on improving patient adherence to infection control practices in various environments, including schools, family households, and local communities.

Additionally, individuals visiting regions where shigellosis is endemic should receive education on preventive strategies, including practicing proper hand hygiene, ensuring access to clean drinking water, and adopting safe food consumption practices.

Notes

Ethics Approval

The collection of data in accordance with Article 18 of the infectious Disease Control and Prevention Act was approved by the Institutional Review Board of the KDCA (KDCA: 2023-09-04-PE-01).

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: all authors; Data curation: YA, JP; Formal analysis: SJ, ES, JK, JY; Investigation: SJ, YA, JP, HL, HL; Methodology: YA, JP, HL, HL; Project administration: YAG; Resources: SJ; Supervision: YAG; Visualization: YA; Writing–original draft: YA; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions

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Original Article

Living arrangements and metabolic syndrome: a national cross-sectional study in the Republic of Korea

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ABSTRACT

Objectives: This study investigated the relationship between living arrangements and metabolic syndrome (MetS) risk in the adult population in the Republic of Korea.
Methods: The samples were derived from the data collected during the second year of the seventh Korea National Health and Nutrition Examination Survey. The study targeted a total of 6,265 adults who were aged 20 years and above, and multiple logistic regression analysis was conducted. Living arrangements were classified into 4 categories: single-person households, 1-generation households, 2-generation households, and other family types. MetS was identified by the presence of at least 3 out of the 5 National Cholesterol Education Program Adult Treatment Panel III criteria.
Results: For men, the odds ratio (ORs) for MetS in 1- and 2-generation households, compared to single-person households, were 0.92 (95% confidence interval [CI], 0.55–1.54) and 0.97 (95% CI, 0.58–1.62), respectively. The OR for other types of households was 0.96 (95% CI, 0.79–1.17). For women, the OR for MetS in 1- and 2-generation households, compared to single-person households, were 1.52 (95% CI, 1.15–2.01) and 1.29 (95% CI, 1.01–1.67), respectively.
Conclusion: Our study suggests that a national strategy involving tailored interventions for women living in high-risk conditions is necessary to reduce the risk of MetS in Korean women.

Keywords: Living arrangement; Metabolic syndrome; Socioeconomic status

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Introduction

Metabolic syndrome (MetS), which comprises a cluster of risk factors for cardiovascular and disease and all-cause mortality, is a global health problem [1]. In the Republic of Korea, the prevalence of MetS rose from 1.53% in 2008 to 3.19% in 2017 [2].

Recent studies have reported that genetic variations and shared environmental factors contribute to the heritability of MetS, as evidenced by familial correlations [3]. Some research has explored the relationship between familial ties and environmental factors, including

lifestyle changes, climate, geography, and migration [4]. The advantages and disadvantages of different living arrangements, such as living alone or living with children, have also been discussed [5]. From another angle, a connection has been observed between single-child families and cardio-metabolic risk factors [6]. There is a growing interest in the relationship between MetS, marital status, and living arrangements, with numerous studies investigating the link between social and economic conditions and MetS [7–9].

From the standpoint of familial aggregation, the structure of a family has been identified as a significant predictive factor for maternal and paternal connections [10]. Marriage, in particular, encourages healthy behavior and bolsters mental health, thereby serving as a crucial source of social support that contributes to physical well-being. Furthermore, marriage can exert social control over lifestyle habits that may be detrimental to one's health [11–13].

Due to shifts in economic structure and development, the traditional extended family structure in Korea has recently transitioned into a nuclear family structure. Consequently, the number of single-generation families has significantly increased [14,15]. These alterations in family structure are transforming adult lifestyles, thereby significantly influencing their healthcare issues [7,16,17].

Previous studies have examined the associations of living arrangements with MetS [18–22] and psychological health [23–26]. Studies have also concentrated on the correlation between socioeconomic status and MetS in middle-aged and elderly people [5,9,10,12,27]. Despite the numerous studies on MetS, there is a dearth of research examining the relationship between living arrangements and the incidence of MetS. Consequently, this study aims to investigate the connection between living arrangements and the risk of MetS in the Korean adult population, with a focus on gender differences. The data for this study was sourced from the Korea National Health and Nutrition Examination Surveys (KNHANES), conducted by the Korea Disease Control and Prevention Agency.

HIGHLIGHTS

- This study investigated the relationship between living arrangements and metabolic syndrome risk in the adult population in the Republic of Korea, using the Korea National Health and Nutrition Examination Surveys conducted by the Korea Disease Control and Prevention Agency.
- This study suggests that a national strategy involving customized interventions for women with risky living arrangements should be pursued to decrease the risk of metabolic syndrome in Korean women.

Materials and Methods

Study Participants

This study utilized data from the second year of the KNHANES VII (2017), an annual survey that includes a health examination, health interview, and nutrition survey. The survey employs a stratified multistage cluster sampling method to draw a representative sample from the non-institutionalized civilian population of the Republic of Korea. Participants with missing data for at least 1 variable related to MetS were excluded from the study. Ultimately, the study included 6,265 participants. All participants provided informed consent to participate in the survey.

Demographic Characteristics, Anthropometric Variables, and Living Arrangement

For the health interview survey, we chose demographic variables such as age, gender, residential area, physical activity, alcohol consumption, smoking status, and self-perceived health condition. We selected education level and household income level as socioeconomic indicators. Additionally, we used body mass index (BMI) and waist circumference (WC) as anthropometric variables.

The area of residence was categorized into rural and urban. The definition of a rural residence was provided in the health interview survey. Physical exercise was divided into non-exercise and regular exercise. The regular exercise category included individuals who exercised 3 or more times a week, with each session lasting more than 20 minutes. Alcohol consumption was split into 3 categories: non-drinkers, mild-moderate drinkers, and heavy drinkers. Individuals who consumed 3 or more drinks per day were classified as heavy drinkers, while those who consumed alcohol once or more a month were simply classified as drinkers. Smoking status was divided into 2 categories: non-smokers and current smokers. A current smoker was defined as an individual who had smoked 100 or more cigarettes and was still smoking. The non-smoker category included former smokers. Education level was divided into 4 categories: elementary school, middle school, high school, and university. Household income level was divided into 4 categories based on quartiles: lowest, middle-low, middle-high, and highest. WC was measured at the narrowest point between the lower border of the rib cage and the iliac crest. The formula for calculating BMI is as follows: weight (kg)/

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height squared (m^2). Living arrangements were divided into 4 groups: (1) single-person households, adults living alone; (2) 1-generation households, adults living with a spouse; (3) 2-generation households, adults living with children; (4) others, adults living with grandparents and other relatives.

Definitions of MetS

According to the updated National Cholesterol Education Program Adult Treatment Panel III criteria, MetS is defined by the presence of 3 or more of the following 5 criteria: (1) a WC of 90 cm or more in men, or 80 cm or more in women, as per the International Obesity Task Force standards for the Asian-Pacific population; (2) a blood pressure (BP) reading of 130/85 mmHg or higher, or the use of antihypertensive medication; (3) a fasting blood glucose level of 100 mg/dL or higher, or the use of medication such as insulin or oral agents; (4) a triglyceride level of 150 mg/dL or higher, or the use of medication; and (5) a high-density lipoprotein (HDL) cholesterol level less than 40 mg/dL in men or less than 50 mg/dL in women, or the use of medication.

Statistical Analysis

We utilized the SAS survey procedure ver. 9.2 (SAS Institute Inc.) for our statistical analysis. To compare differences in anthropometric, laboratory, and demographic variables based on gender, we employed either the t-test or the chi-square test. We used the chi-square test to compare the prevalence of MetS and each of its components according to different living arrangements. Multiple logistic regression analyses were conducted to assess the risk of MetS as an independent variable related to living arrangements. In these analyses, the reference group for living arrangements was single-person households.

Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of MetS were calculated for different groups: single-person households, 1-generation households, 2-generation households, and others. We examined the changes in the OR for MetS risk in each living arrangement, adjusting for age and BMI in model 1. In model 2, we further adjusted for exercise, alcohol consumption, smoking habits, and self-reported health condition. Finally, in model 3, we adjusted for the covariates in model 2, as well as household income and education level. A 2-sided *p*-value of less than 0.05 was deemed statistically significant.

Results

Table 1 shows the general characteristics of the 6,265 study participants according to gender. The average age of men was 41 years, and that of women was 44 years. The average age for men was 41 years, while for women it was 44 years. The distribution of living arrangements for men was as follows: 2-generation households, 61.8%; 1-generation households, 28.0%; single-person households, 5.4%; and other arrangements, 4.8%. For women, the distribution was as follows: 2-generation households, 57.7%; 1-generation households, 28.4%; single-person households, 8.2%; and other arrangements, 5.7%. Both household income and education level were higher among men than women. Men also smoked and consumed alcohol much more

Table 1. General characteristics of study participants according to gender

Characteristic	Man (*n* = 2,820)	Woman (*n* = 3,445)	*p*[a]
Age (y)	41.6 ± 0.3	44.4 ± 0.3	< 0.001
Residence area			0.35
Rural	19.8 (1.7)	19.3 (1.7)	
Exercise			< 0.001
Yes	24.1 (0.6)	16.9 (0.5)	
Alcohol drinking			< 0.001
Non-drinker	23.1 (0.6)	38.9 (0.6)	
Mild-to-moderate drinker	61.4 (0.7)	59 (0.6)	
Heavy drinker	15.5 (0.5)	2 (0.2)	
Smoking			< 0.001
Current smoker	57.8 (0.7)	8.7 (0.3)	
Self-health condition			< 0.001
Good	39.9 (1.3)	40.1 (1.4)	
Moderate	35.3 (0.7)	35.2 (0.8)	
Bad	24.8 (1.3)	24.7 (1.3)	
Education			< 0.001
Elementary school or less	17.8 (0.5)	28 (0.7)	
Middle school	13.6 (0.5)	13.2 (0.5)	
High school	37 (0.7)	32.9 (0.7)	
College and higher	31.5 (0.7)	25.9 (0.7)	
Income			< 0.001
Lowest	13.2 (0.6)	17 (0.6)	
Medium-lowest	26.8 (0.8)	28.2 (0.8)	
Medium-highest	30.7 (0.8)	28.2 (0.7)	
Highest	29.3 (0.9)	26.7 (0.8)	
Living arrangement			< 0.001
Single	5.4 (0.7)	8.2 (0.7)	
One-generation	28.0 (0.6)	28.4 (0.6)	
Two-generation	61.8 (0.8)	57.7 (0.8)	
Others	4.8 (0.4)	5.7 (0.3)	
BMI (kg/m^2)	23.7 ± 0.1	22.9 ± 0.1	< 0.001
WC (cm)	82.4 ± 0.2	76.7 ± 0.2	< 0.001
BP (mmHg)	123.8 ± 16.5	122.4 ± 18.3	< 0.001
Fasting glucose (mg/dL)	105.5 ± 27.2	100.9 ± 24.5	< 0.001
TG (mg/dL)	163.9 ± 133.5	131 ± 88.2	< 0.001
HDL cholesterol (mg/dL)	45.9 ± 11.2	50.3 ± 11.7	< 0.001

Data are presented as mean ± SE or % (SE)
BMI, body mass index; WC, waist circumference; BP, blood pressure; TG, triglyceride; HDL, high-density lipoprotein; SE, standard error.
[a]Calculated using the Student t-test or the chi-square test.

frequently than women, despite exercising more regularly. Furthermore, men exhibited higher levels of BMI, WC, BP, fasting glucose, and triglyceride compared to women.

Table 2 illustrates the univariate relationship between living arrangements and MetS, along with its components, differentiated by gender. The incidence of MetS in single-person households was 24.6% for men and 45.3% for women. In 1-generation households, the prevalence was 30.9% for men and 40.2% for women. In 2-generation households, the rates were 20.9% for men and 17.3% for women. In other types of households, the prevalence was 7.1% for men and 9.9% for women. For both genders, a larger living arrangement corresponded to a lower prevalence of MetS ($p < 0.001$). The prevalence of each of the 5 MetS components showed a significant variation based on living arrangement ($p < 0.001$). For men, a larger living arrangement was associated with a lower proportion of high BP, high blood glucose, high triglycerides, and low HDL cholesterol, with the exception of abdominal obesity. For women, a larger living arrangement corresponded to a lower proportion of abdominal obesity, high BP, high blood glucose, high triglycerides, and low HDL cholesterol ($p < 0.001$).

Table 3 depicts the ORs for MetS across different living arrangements, with the single-person household group serving as the reference. For men, the adjusted ORs for the 1-generation, 2-generation, and other categories compared to the single-person household were 0.92 (95% CI, 0.55–1.54), 0.97 (95% CI, 0.58–1.62), and 0.96 (95% CI, 0.79–1.17), respectively, in model 3. For women, the adjusted OR for the 1-generation category compared to single-person households was 1.34 (95% CI, 1.07–1.69; $p < 0.001$) in model 1. When additional behavioral risk factors (alcohol, smoking, and exercise) were

Table 2. Prevalence of metabolic syndrome and each component according to living arrangement by gender

Variable	Single	One-generation	Two-generation	Others	*p* for trend[a]
Man					
Metabolic syndrome	24.6 (2.7)	30.9 (1.3)	20.9 (0.7)	7.1 (0.7)	<0.001
Abdominal obesity	22.5 (3.1)	25.6 (1.3)	21.3 (0.8)	5.5 (0.9)	0.0613
High blood pressure	40.4 (3.3)	52.5 (1.5)	34.7 (0.9)	8.5 (1.1)	<0.001
High fasting glucose	31.8 (3.1)	36.5 (1.3)	24.4 (0.8)	10.1 (0.8)	<0.001
High triglyceride	41.1 (3.4)	39.7 (1.4)	33.1 (0.8)	7.9 (1.1)	<0.001
Low HDL cholesterol	22.6 (3.0)	26.1 (1.4)	18.8 (0.7)	6.1 (0.9)	<0.001
Woman					
Metabolic syndrome	45.3 (2.4)	40.2 (1.3)	17.3 (0.6)	9.9 (0.5)	<0.001
Abdominal obesity	51.0 (2.6)	50.2 (1.5)	30.9 (0.7)	8.2 (0.6)	<0.001
High blood pressure	54.6 (2.6)	48.0 (1.5)	20.1 (0.7)	8.6 (0.4)	<0.001
High fasting glucose	35.4 (2.2)	30.6 (1.2)	15.4 (0.6)	12.7 (0.6)	<0.001
High triglyceride	35.1 (2.3)	34.9 (1.3)	18.5 (0.6)	8.4 (0.7)	<0.001
Low HDL cholesterol	46.1 (2.4)	46.9 (1.3)	32.6 (0.8)	12.2 (1.1)	<0.001

Data are presented as % (standard error).
HDL, high-density lipoprotein.
[a]Calculated using the chi-square test.

Table 3. Adjusted odds ratios and 95% confidence intervals for metabolic syndrome according to living arrangement

Variable	Model 1[a]	Model 2[b]	Model 3[c]
Man			
Single	1.00	1.00	1.00
One-generation	0.88 (0.57–1.36)	1.02 (0.65–1.60)	0.92 (0.55–1.54)
Two-generation	1.04 (0.70–1.56)	1.13 (0.74–1.73)	0.97 (0.58–1.62)
Others	1.01 (0.82–1.22)	0.96 (0.79–1.17)	0.96 (0.79–1.17)
Woman			
Single	1.00	1.00	1.00
One-generation	1.34 (1.07–1.69)	1.44 (1.14–1.82)	1.52 (1.15–2.01)
Two-generation	1.10 (0.86–1.40)	1.33 (0.98–1.79)	1.29 (1.01–1.67)
Others	1.04 (0.89–1.22)	1.03 (0.88–1.22)	1.06 (0.90–1.25)

Adjusted weighted regression analysis. All values are odds ratios with 95% confidence intervals.
[a]Adjusted for age and body mass index. [b]Additionally adjusted for exercise, alcohol, smoking, and, self-health condition. [c]Additionally adjusted for education and income.

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adjusted for, the OR increased to 1.44 (95% CI, 1.14–1.82; $p < 0.001$) in model 2. Further adjustment for socioeconomic factors (household income and education level) resulted in an OR of 1.52 (95% CI, 1.15–2.01; $p < 0.001$) in model 3. The OR for MetS in the 2-generation category compared to single-person households was 1.29 (95% CI, 1.01–1.67; $p < 0.001$) after adjusting for all components. However, the OR for the "other" category compared to single-person households was not statistically significant.

Discussion

This study investigated the association between living arrangements and the risk of MetS in the adult population of the Republic of Korea, using data from the second year of the KNHANES VII. The findings indicated a downward trend in the adjusted ORs for 1- and 2-generation households, as well as other living arrangements, compared to single-person households among men. Conversely, there was an upward trend in the adjusted ORs for 1- and 2-generation households, and other living arrangements, compared to single-person households among women.

Significant gender differences were revealed in the association between living arrangements and the risk of MetS. A similar gender-specific association was found in another Chinese study, which attributed the risk of MetS to various factors such as age, physical activity, and education level. Some of these findings align with ours, even though living arrangements were not considered in that study [28]. Previous research has reported familial correlations with MetS, which supports our findings. These studies suggest that both shared genetic and environmental factors contribute to the risk of MetS, reinforcing the importance of considering living arrangements [3].

There is compelling evidence from a previous cohort study suggesting a gender difference in the relationship between markers of MetS and an elevated risk of cardiovascular disease [29]. In our study, the higher prevalence of MetS in women than in men could be partially explained by another study's findings, which indicated an increase in MetS in women following menopause [19].

Our study showed that a 2-generation living arrangement appeared to reduce the risk of MetS more significantly in women than a 1-generation arrangement. To the best of our knowledge, this is the first study to investigate the relationship between MetS and different living situations, with a specific focus on gender differences.

Living in a household with a spouse and children has been reported to provide health benefits [5]. These findings have been partially attributed to the shared environment and familial correlations. More specifically, in men, the risk of MetS was found to be higher in single-person households compared to those living with a spouse in a 1-generation family. However, this effect was moderated by factors such as income and education level. In the case of women, obesity was identified as a significant issue, with socio-demographic factors and lifestyle behaviors serving as explanatory variables [30].

Our study's findings regarding gender differences in MetS are corroborated by another study, which also identified gender disparities in MetS and its associated factors in Taiwan [10].

Our study has several strengths. Firstly, we incorporated a variety of living arrangements to reflect the recent shifts in living trends. Secondly, we noted gender-based differences, where distinct lifestyles emerged as the pivotal factor linking MetS to various living arrangements.

In conclusion, this study determined that individuals living with family members, particularly with a spouse, have an increased risk of MetS compared to those living alone. This risk is especially pronounced among women. Our study indicates that it is necessary to implement a national strategy involving interventions tailored for women with high-risk living conditions to reduce the risk of MetS in Korean women. Additional prospective studies should be conducted to further develop these findings and investigate the mechanisms that promote sustainable strategies and management of MetS.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare. Aeree Sohn was not involved in the editorial process or review of this manuscript, despite being a member of the Editorial Board of the Osong Public Health and Research Perspectives.

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

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Short Communication

Characteristics of a large outbreak arising from a school field trip after COVID-19 restrictions were eased in 2022

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ABSTRACT

Objectives: This study analyzed a large outbreak of coronavirus disease 2019 (COVID-19) that occurred during a high school field trip in the Jeonbuk region and aimed to identify risk factors for COVID-19 infection, with the goal of preventing such outbreaks in the future.
Methods: A retrospective cohort study of 737 participants, including 668 students and 69 staff at High School A, was designed to describe the epidemiological characteristics of this large COVID-19 outbreak. Logistic regression analysis was performed to calculate relative risks (odds ratios [ORs]) and 95% confidence intervals (CIs).
Results: There were 190 confirmed cases (174 students, 16 staff), with an attack rate of 25.8%. Small outbreaks were decreasing before the field trip, but this trend reversed after the trip, leading to larger outbreaks. Logistic regression showed an OR of 2.39 (95% CI, 1.66–3.43; $p < 0.05$) for COVID-19 infection among field trip participants. Among them, 11th graders had an OR of 2.32 (95% CI, 1.53–3.52; $p < 0.05$) compared to 10th graders, while no significant risk difference was found within same-grade teams.
Conclusion: There was a high risk for COVID-19 transmission during extracurricular activities with a large number of participants, such as field trips, even after the nationwide Omicron variant epidemic subsided. Even when students are separated into teams and follow different routes, it is challenging to design routes that entirely prevent contact between teams. Thus, programs should be designed carefully, and students with symptoms should be identified before and during the program to isolate them promptly.

Keywords: COVID-19; Large outbreak; School field trips

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Introduction

After the end of January 2022, the number of confirmed coronavirus disease 2019 (COVID-19) cases in the Republic of Korea began to increase with the emergence of the Omicron variant. The cases peaked in March and then started to decline gradually after April [1]. In response to this outbreak, Korean public health authorities initiated Phase 1 preparations for a return

to normal life, which involved easing measures such as social distancing, starting from April 18, 2022 [2]. From May 2022, schools were given the option to switch back to in-person classes from the online learning routine that had been in place to prevent large COVID-19 outbreaks within educational settings. With the resumption of in-person classes, schools also restarted overnight programs, such as field trips [3]. In addition, schools developed COVID-19 prevention and management guidelines in collaboration with public health authorities. These guidelines aimed to establish an effective infection management system, which included monitoring for symptomatic individuals and the use of rapid self-test kits, to support a stable transition back to normal life [4].

Despite these efforts, however, a large outbreak took place among 431 individuals (404 students and 27 staff) who participated in a school field trip at High School A in Jeollabuk-do in June 2022, with 139 confirmed cases. High School A is a girls' high school with a total enrollment of 737 students and staff (668 students and 69 staff members). The field trip was a 4-day event that took place from July 12 to July 15, 2022, in the Jeju Special Self-Governing Province. The attendees included 215 10th graders, 189 11th graders, and 27 teachers. Typically, the school monitored and tested individuals who showed symptoms to prevent and control the spread of infectious diseases. Confirmed cases were isolated to prevent further transmission within the school. On July 11, the day before the field trip, rapid antigen tests were administered to all participants. The field trip was organized into 4 teams (2 teams per grade), and each class traveled on designated buses. Furthermore, individuals exhibiting symptoms during the field trip were checked and tested daily. Those who tested positive were promptly sent home under staff supervision.

In this study, we aimed to identify risk factors in a large-scale outbreak that occurred during a school field trip when COVID-19 control measures were being eased. We seek to propose strategies and measures to prevent and manage such outbreaks in similar situations in the future.

HIGHLIGHTS

- There was a high risk for COVID-19 transmission during extracurricular activities with a large number of participants, such as field trips, even after the Omicron outbreak subsided.
- Even when students are divided into teams and follow distinct routes, designing paths that entirely prevent interactions between teams is challenging. Therefore, students still encounter each other closely during meals and in their accommodations.
- Programs should be designed carefully, and students with symptoms should be identified before and during the program in order to quarantine them promptly.

Materials and Methods

Study Population and Case Definition

The study population comprised 737 students and staff of High School A (668 students, 69 staff), where a large COVID-19 outbreak took place after a school field trip in July 2022.

Cases at High School A were defined for the period from June 28, 2022, to July 21, 2022, using the following criteria: (1) detection of the COVID-19 gene via polymerase chain reaction or isolation of the virus, or (2) a positive result on a rapid antigen test administered by healthcare providers [5]. These criteria were in line with the diagnostic testing guidelines provided in the COVID-19 Response Guidelines. Applying this definition, a total of 190 cases were confirmed [5].

Study Design

A retrospective cohort design was utilized. Data were collected using an epidemiological survey questionnaire distributed to all students and staff. This questionnaire captured personal information, including grade, class, sex, age, participation in a field trip, the date of symptom onset, and the types of initial symptoms. Additional data were obtained from the school health administrator, which provided detailed information about the field trip, such as team divisions, bus boarding logistics, and the itinerary of locations visited at specific times. The Korea Disease Control and Prevention Agency's COVID-19 Information System was utilized to confirm individual-level COVID-19 diagnosis statuses. This information was instrumental in defining the cases for our study.

The collected data were statistically analyzed and described to investigate the progression of the COVID-19 outbreak at High School A and the risk factors for infection during this large outbreak.

Statistical Analysis

The epidemic curve was constructed based on the time of onset of the first symptom. Among the 190 confirmed cases, 176 individuals were symptomatic (with 14 being asymptomatic); these cases were categorized by whether they were students or teachers, and by their attendance at a school field trip.

In assessing the incidence rate and risk factors, 2 separate

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analyses were conducted: one encompassing the entire study population and another focusing specifically on school trip attendees. Risk factors were subjected to statistical analysis and the results were expressed as odds ratios (ORs), adjusted for student/teacher status and sex, along with 95% confidence intervals (CIs).

To manage and visualize the data, Microsoft Excel ver. 2013 (Microsoft Corp.) was used, and statistical analyses were performed using the R ver. 3.6.3 (The R Foundation).

Ethics Review

This study was approved by the Institutional Review Board (IRB) of the Korea Disease Control and Prevention Agency (IRB No: KDCA-2023-07-03-PE-01).

Results

Attack Rate

Of the 737 students and staff at High School A, 190 met the definition of a case, resulting in an attack rate of 25.8%. Of the 190 cases, 174 were students, accounting for 91.6% of the cases (attack rate, 26.0%). Among student groups, 10th and 11th graders had the greatest number of cases (63 and 88, respectively), and accounted for 79.5% of the student cases. The highest attack rate was found among 11th graders (41.9%). Of all cases, 139 were attendees of the school field trip; in this group, the attack rate was 32.3%, which was 2-fold higher than that among non-attendees (Table 1).

The symptoms reported among confirmed cases included sore throat (*n*=100, 52.6%), fever (*n*=79, 41.6%), cough (*n*=76, 40.0%), headache (*n*=33, 17.4%), and phlegm (*n*=31, 16.3%). There were no cases of severe symptoms necessitating hospitalization (Table 2).

Epidemic Curve

There were 2 distinct waves: the first from June 28 to July 12, 2022, and the second starting after the school field trip. The first outbreak predominantly affected 10th graders, 12th graders, and staff (since the index case was diagnosed on June 28, 2022), peaking on July 10, 2022, with 14 confirmed cases, after which it subsided.

The second outbreak began with 4 confirmed cases on the day of the school field trip on July 12, 2022. It peaked on July 16, 2022, and eventually began to slowly subside. During the second outbreak, there was an increase in the number of confirmed cases as a result of close contact with a confirmed patient attending the school trip. Most of the confirmed cases during the second outbreak were attendees of the school trip, and the second outbreak was larger than the first due to trip-specific risk factors, such as traveling in the same bus and

Table 1. General characteristics and attack rate in a COVID-19 outbreak in 2022

Characteristic	Total (*n*=737)	Case (*n*=190)	Non-cases (*n*=547)	Attack rate (%)
Age group (y)				
0–19	678 (92.0)	174 (91.6)	504 (92.1)	25.7
20–39	20 (2.7)	6 (3.2)	14 (2.6)	30.0
≥40	39 (5.3)	10 (5.3)	29 (5.3)	25.6
Sex				
Male	25 (3.4)	5 (2.6)	20 (3.7)	20.0
Female	712 (96.6)	185 (97.4)	527 (96.3)	26.0
Identity				
Student	668 (90.6)	174 (91.6)	494 (90.3)	26.0
Staff	69 (9.4)	16 (8.4)	53 (9.7)	23.2
Grade level[a]				
10th grade	236 (32.0)	63 (33.2)	173 (31.6)	26.7
11th grade	210 (28.5)	88 (46.3)	122 (22.3)	41.9
12th grade	222 (30.1)	23 (12.1)	199 (36.4)	10.4
School trip attendance				
Non-attendee	306 (41.5)	51 (26.8)	255 (46.6)	16.7
Attendee	431 (58.5)	139 (73.2)	292 (53.4)	32.3
Team				
10th grade team 1	125 (17.0)	25 (13.2)	100 (18.3)	20.0
10th grade team 2	103 (14.0)	29 (15.3)	74 (13.5)	28.2
11th grade team 1	113 (15.3)	49 (25.8)	64 (11.7)	43.4
11th grade team 2	90 (12.2)	36 (18.9)	54 (9.9)	40.0

Data are presented as *n* (%) unless otherwise stated.
[a]Excluding staff (*n*=69).

staying in the same accommodation (Figure 1).

Risk Factors

Among all students and staff at High School A, the OR was 2.39 among school trip attendees (95% CI, 1.66–3.43; $p < 0.05$) compared to non-attendees (Table 3).

Among the 10th and 11th graders who attended the school trip, the number of cases was 1.5 times higher among 11th graders than among 10th graders, with a statistically significant OR of 2.32 (95% CI, 1.53–3.52; $p < 0.05$). However, there were no differences among teams within the same grade level (Table 4).

During the school trip, there were 4 different teams (2 teams in each grade level), and the duration of their contact was 34.2 hours for 10th graders and 32.8 hours for 11th graders. The duration of contact between grade levels was 4.8 hours (Figure 2).

Table 2. Frequency and percentage of COVID-19 symptoms (overlapping)

Symptom	Value ($n = 190$)
Sore throat	102 (53.7)
Fever	80 (42.1)
Cough	76 (40.0)
Headache	33 (17.4)
Phlegm	31 (16.3)
Chills	12 (6.3)
Muscle pain	9 (4.7)
Runny nose	8 (4.2)
Stuffed nose	5 (2.6)
Diarrhea	3 (1.6)
Other[a)]	8 (4.2)
Asymptomatic	14 (7.4)
Unknown	17 (8.9)

Data are presented as *n* (%).
[a)]Abdominal pain, loss of smell, hoarse voice, dizziness, loss of taste, dyspnea, chest pain, and vomiting (1 case each).

Discussion

In response to the influx and spread of COVID-19 in 2020, educational authorities recommended that schools suspend in-person classes and transition to virtual learning from the first semester of 2020 [6]. However, to address the challenges of maintaining strict disease control and promoting sustainable COVID-19 responses amid the prolonged pandemic, authorities permitted schools to develop their own management systems starting in the second semester of 2022. This included the option to transition back to in-person classes.

The present study analyzed the characteristics and risk factors of a large outbreak that occurred as schools began

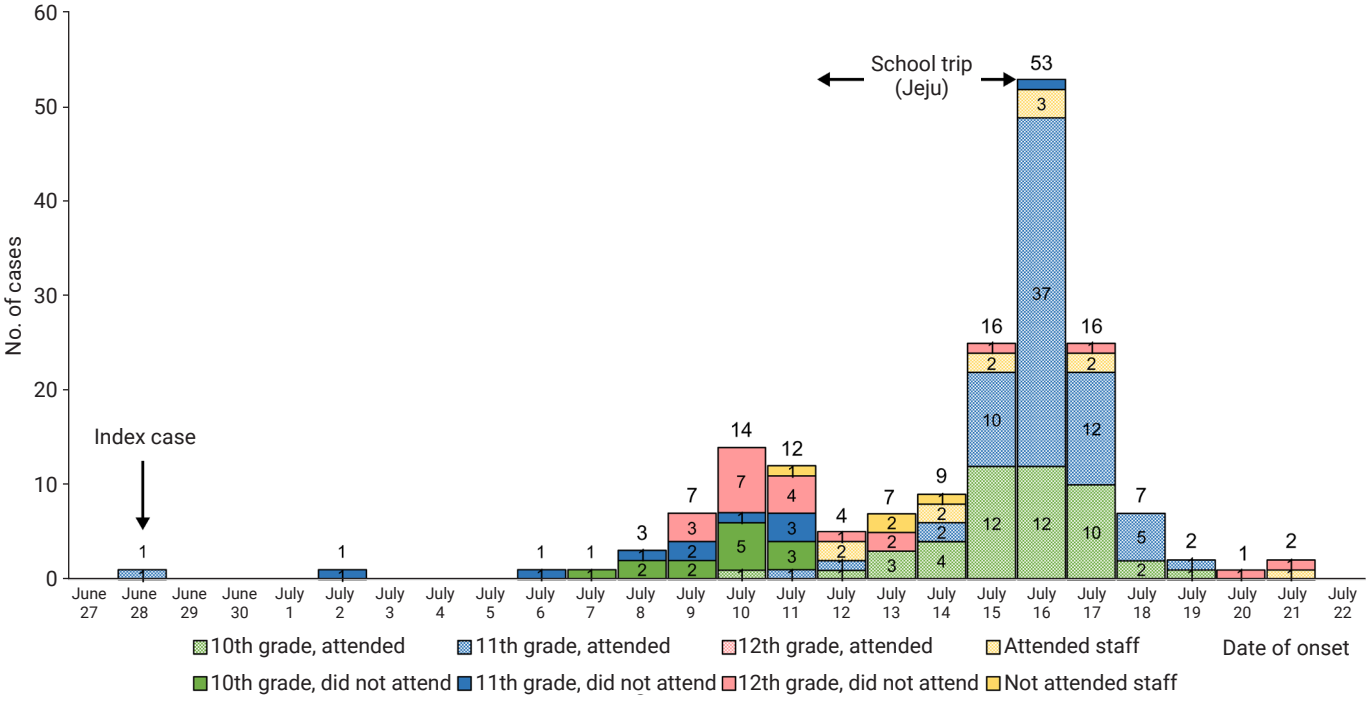

Figure 1. Number of symptomatic cases by date of symptom onset, school trip attendance, and grade level or staff status.

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Table 3. Risk factors among all COVID-19 cases

Category	Total (*n* = 737)	Case (*n* = 190)	Non-case (*n* = 547)	aOR (95% CI)	*p*[a]
Sex					
Male	25	5	20	Ref.[c]	-
Female	712	185	527	1.36 (0.41–4.59)	0.62
Identity					
Staff	69	16	53	Ref.[c]	-
Students	668	174	494	0.97 (0.53–1.77)	0.93
School trip attendance[b]					
Non-attendee	306	51	255	Ref.[c]	-
Attendee	431	139	292	2.39 (1.66–3.43)	< 0.05

The age group variable was excluded from the adjusted model due to convergence issues caused by multicollinearity.
aOR, adjusted odds ratio; CI, confidence interval; ref., reference; -, This table presents the results of logistic regression analysis based on sex, social status, and participation in school trips among all participants, suggesting the possibility that participating in the school trip was a risk factor.
[a]Determined using logistic regression analysis. [b]School trip attendees were students (10th and 11th graders) and staff. [c]Reference represents the value indicating the reference level (= 0) for the respective row.

Table 4. Risk factors among school trip attendees

Category	Total (*n* = 431)	Case (*n* = 139)	Non-case (*n* = 292)	aOR (95% CI)	*p*[a]
Sex					
Male	10	4	6	Ref.[c]	-
Female	421	135	286	1.4 (0.27–7.16)	0.69
Identity					
Staff	27	12	15	Ref.[c]-	-
Student	404	127	277	0.52 (0.19–1.42)	0.2
Grade level[b]					
10th grade	228	54	174	Ref.[c]	-
11th grade	203	85	118	2.32 (1.53–3.52)	< 0.05

aOR, adjusted odds ratio; CI, confidence interval; ref., reference; -, This table displays the results of logistic regression analysis for school trip participants, categorized by sex, social status, and grade level. The results suggest that there were differences in risk factors among different grades.
[a]Determined using logistic regression analysis. [b]Including staff who chaperoned each grade level. [c]Reference represents the value indicating the reference level (= 0) for the respective row.

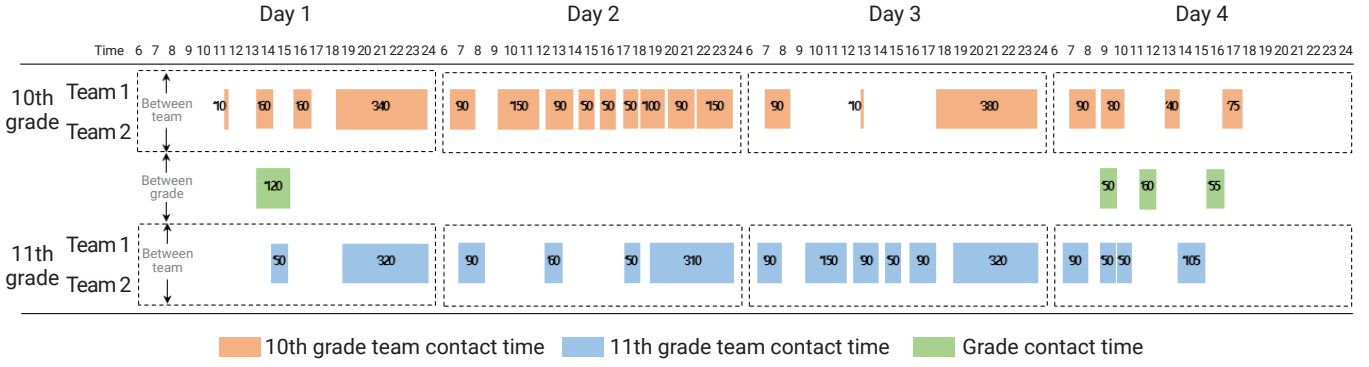

Figure 2. Duration of contact at field trip sites between grade levels and teams. Each color denotes a field trip site, and the width of the box denotes the duration of time spent at the corresponding site (minutes).

to ease their control measures and resume school-wide extracurricular programs. Based on the findings, the following conclusions were drawn regarding important considerations when transitioning into phases where control measures are relaxed.

In response to the first outbreak at High School A before the school trip, High School A minimized path-crossing between classes and limited after-school programs. From June 30 to July 6, 2022, the school was conducting midterms, which resulted in only morning classes being held, thereby

reducing contact among students. Consequently, from the time the index case was diagnosed until just before the school trip, a total of 35 cases were confirmed. During the subsequent outbreak, from July 12 to July 21, 2022, there were 155 confirmed cases, indicating that 81.6% of the total cases occurred in this second wave. The significant increase in cases during this period is largely attributed to the students spending extended periods together in enclosed spaces, such as buses, and staying in shared accommodations.

Despite confirmed cases of COVID-19 within the school prior to the school trip, the school decided to proceed with the trip to avoid the high costs associated with canceling prearranged accommodations and restaurant bookings. However, a more stringent management of close contacts of COVID-19 patients could have reduced the spread of the virus. Nonetheless, it is a daunting task for a single health care provider to manage all aspects of infection prevention and control in a school of 737 individuals. Therefore, it is essential to determine the optimal provider-to-individual ratio to ensure adequate staffing or resource allocation. This would enable comprehensive testing of all potential participants before a large event, such as a school trip, and the exclusion of those with confirmed infections [7].

During the school trip, an issue that arose was the difficulty in preventing teams from crossing paths, despite plans to minimize such occurrences. Space limitations made complete separation unfeasible. Additionally, the use of shared spaces like restaurants and accommodations heightened the risk of exposure in enclosed, crowded settings, which is particularly problematic for respiratory illnesses. However, the study did not monitor specific exposure scenarios for each team, complicating the task of providing a definitive explanation for the markedly different ORs observed across various grade levels. Despite these challenges, the data showed that teams sharing the same routes for prolonged periods had similar attack rates. To mitigate these risks, schools should consider organizing trips so that different grade levels visit separate regions or scheduling trips during distinct seasons, such as spring and fall [8].

At events where many attendees are expected, it is imperative to note that prevention is the best strategy for management. When planning activities such as school trips off-campus during the ongoing spread of COVID-19, it is important not only to monitor individuals for symptoms but also to screen close contacts to identify potential asymptomatic carriers in the incubation period. Should the school nurse be unable to make these determinations, schools are advised to consult with local epidemiological investigators. Additionally, an effective infection prevention and response system should be established. This system should include instructing attendees to promptly inform the chaperone if they experience symptoms and to rigorously maintain personal hygiene practices. Chaperones, in turn, must regularly check attendees for symptoms and be well-versed in the management manual to effectively handle any cases that arise.

Notes

Ethics Approval

This study was approved by the Institutional Review Board of the Korea Disease Control and Prevention Agency (IRB No: KDCA-2023-07-03-PE-01).

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: all authors; Data curation: SJK, EYK; Formal analysis: SJK; Investigation: SJK, EYK; Methodology: JY, SJK; Project administration: JY; Resources: JY; Software: SJK; Supervision: JY; Validation: JY; Visualization: SJK; Writing–original draft: SJK; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions

Korea Disease Control and Prevention Agency provided statistical support and the photographs that constitute Figure 1.

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Commentary

Activities of the Republic of Korea in the Global Health Security Agenda

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The Ebola virus outbreak that began in Guinea, West Africa, in December 2013 eventually spread to several other countries, including Liberia, Sierra Leone, Nigeria, Senegal, Mali, the United States, and Spain. This prompted a consensus regarding the need for a new global health cooperative to enhance global health security. There was also criticism regarding the initial response by the World Health Organization (WHO), which many considered inadequate. In response to these concerns, the Global Health Security Agenda (GHSA) was launched in December 2014. This initiative was spearheaded by the United States, alongside 27 other countries and international organizations, including the WHO, the World Organization for Animal Health, and the Food and Agriculture Organization of the United Nations [1].

Through multilateral and multi-sectoral partnerships, the GHSA strives to create a world safe from the threat of infectious diseases [2]. Its goal is to protect human life and minimize social and economic losses by preventing, detecting, and responding to pathogens that occur naturally, or are accidentally or intentionally released. To achieve this, the GHSA developed 11 Action Packages for prevention, detection, and response during its first phase (2014–2018) and 9 Action Packages for the second phase (2019–2023).

The GHSA operates through Steering Group Meetings for GHSA management and evaluation of the progress of the Action Packages, as well as Action Package Working Group Meetings for discussion between leading countries and member countries on how to conduct and cooperate with each Action Package, implement them in practice, and achieve the predefined goals. Ministerial Meetings are responsible for the vision and future strategy of the GHSA [1].

The following text examines the activities undertaken by the Republic of Korea during the first and second phases to meet the GHSA objectives. It also explores ways to improve health security within the Republic of Korea and among GHSA member nations through the implementation of Action Packages.

The Republic of Korea recognized the significance of enhancing global health security during the Middle East respiratory syndrome (MERS) outbreak in 2015 and has since been an active participant in the GHSA. In 2015, the Republic of Korea hosted the Second GHSA Ministerial Meeting in Seoul, where it announced the Seoul Declaration—the global community's inaugural commitment to health security. Additionally, the Republic of Korea pledged a total of 100 million US dollars over 5 years to strengthen health security capabilities in low-income countries [1]. This meeting was emblematic of the commitment to a multilateral and multi-sectoral cooperative system aimed at

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fortifying global health security.

It is crucial to evaluate each member country's health security capacities and share information with neighboring countries to prevent, detect, and respond promptly and accurately. In 2015, the GHSA conducted a pilot evaluation of health security capacities, focusing on 11 Action Packages, in Uganda, Georgia, Peru, Portugal, Ukraine, and the United Kingdom [1]. The Republic of Korea was involved in the evaluation process for Georgia and Peru. Stemming from this pilot evaluation, the WHO and GHSA introduced the Joint External Evaluation (JEE) in 2016. The JEE is a significant and impactful initiative from the first phase of the GHSA, designed to assess the health security capabilities of member countries.

In 2017, as Chair of the Steering Group, the Republic of Korea conducted the inaugural JEE in the Western Pacific Region of the WHO to facilitate the implementation of JEE. Following the assessment, the Republic of Korea achieved the highest scores among the countries evaluated across 48 categories, securing the maximum of 5 points in 29 categories, 4 points in 15 categories, and 3 points in 4 categories [3]. Leveraging this demonstrated expertise, the Republic of Korea engaged in the Multi-Sectoral Rapid Response, Antimicrobial Resistance, Zoonotic Diseases, Biosafety and Biosecurity, and Immunization Action Packages during the initial phase [1]. At the Second Ministerial Meeting, the Republic of Korea and the United States conducted a joint bio-exercise as part of the Biosafety and Biosecurity Action Package [1]. More recently, at the Fifth Ministerial Meeting, the Republic of Korea presented its National Immunization Program (NIP) to the GHSA member countries, garnering interest and participation from numerous nations.

The GHSA emphasizes multi-sectoral collaboration to achieve its goals. In the academic sector, the Asian Institute for Bioethics and Health Law at Yonsei University wrote a white paper, "South Korean Activities in GHSA from 2014 to 2018 [1]." It established a master's program for local and international students, especially public officials from low-income countries, to strengthen personnel capacities on Action Packages of the GHSA.

In essence, during the first phase of the GHSA, JEE was the main achievement, but sustainable financing capacity remains the main challenge to achieving the goals of each Action Package.

In the second phase of the GHSA, the coronavirus disease 2019 (COVID-19) pandemic necessitated a reassessment of our ability to prevent, detect, respond to, and recover from a Public Health Emergency of International Concern (PHEIC). Drawing on insights from the first phase of the GHSA and the challenges presented by the pandemic, 2 additional Action Packages were introduced: Legal Preparedness and Sustainable Finance. Consequently, the previous 4 Action Packages—Report, Emergency Operations Center, Multi-Sectoral Rapid Response, Medical Countermeasures, and Personnel Deployment—were discontinued. To date, a total of 9 Action Packages have been implemented [2].

HIGHLIGHTS

- The Republic of Korea has achieved remarkable results between 2014 and 2023; it created the Seoul Declaration in 2015 and the New Seoul Declaration in 2022, hosted 2 Ministerial Meetings and played pivotal roles in the Steering Group and Action Packages to lead to the Immunization Action Package and to share the legal preparedness cases from Middle East respiratory syndrome to COVID-19 in the Legal Preparedness Action Package. Also, it has spearheaded the establishment of the Global Health Security Coordinating Office in Seoul as a platform of communication and cooperation with the Global Health Security Agenda members.

The Republic of Korea has taken a leading role in the Immunization Action Package and is also a member of the Legal Preparedness and Sustainable Finance Action Package. The Immunization Action Package is being carried out in collaboration with the People's Republic of Bangladesh, the Kingdom of Saudi Arabia, the United States, and the International Vaccine Institute [2]. The Korea Disease Control and Prevention Agency serves as the secretariat office for the Immunization Action Package, with the secretariat support team based at the Asian Institute for Bioethics and Health Law at Yonsei University.

In the Immunization Action Package, capacities of the NIP during peacetime, such as vaccine management, the mandatory vaccine list, immunization budget, legal framework and policies, mandatory vaccination rate, vaccine registry system, vaccine inventory management system, cold chain logistics, and immunization campaigns, influence the vaccination rate during a PHEIC, like COVID-19. The Republic of Korea has been a leader in achieving high vaccination rates during the COVID-19 pandemic and has shared best practices with the GHSA member countries in the Immunization Action Package working group. The Immunization Action Package Secretariat, along with the secretariat support team, is undertaking a project to conduct literature reviews, including WHO-UNICEF (United Nations International Children's Emergency Fund) data, and to carry out an NIP survey for the 71 GHSA member countries. This project aims

to identify the needs of these countries for expanding their NIP capacities and to establish a cooperative system among GHSA members. Through this initiative, the Immunization Action Package seeks to increase vaccination rates in sustainable ways and to further develop the Immunization Action Package.

As a member of the Legal Preparedness Action Package, the Republic of Korea presented a case study on how legislative preparedness has been used to respond to COVID-19, building on experiences since the MERS outbreak in 2015. Following MERS, the Republic of Korea enhanced and expanded its legal framework to better prevent, detect, and respond to infectious diseases. Improvements included crisis communication, collaboration between central and local governments, the authority of public officials at outbreak sites, equitable distribution of medical resources during a pandemic, transparent disclosure of infectious disease information, and the establishment of an Emergency Operations Center.

The Sustainable Finance Action Package has garnered increased interest from both governmental and non-governmental sectors in the Republic of Korea, with a focus on contributing to the enhancement of health security capacities in low-income countries. There is an intention to collaborate with member countries of the GHSA, international organizations, and non-governmental organizations to fulfill the objectives of the Sustainable Finance Action Package.

The Republic of Korea hosted the Seventh GHSA Ministerial Meeting in Seoul from November 28 to 30, 2022. The meeting commenced with Expert Forums on the Immunization Action Package, Legal Preparedness Action Package, and Sustainable Finance Action Package on the first day. The second day featured the Steering Group Meeting and a side event focused on Disease X. The final day culminated in a Ministerial Meeting where discussions centered on the vision for the GHSA's third phase. This session also included the announcement of the New Seoul Declaration, which underscored the commitment to enhancing capacities for responding to future infectious disease outbreaks, and the declaration of the establishment of the Global Health Security Coordinating Office in the Republic of Korea.

The GHSA saw a significant increase in membership since its inception in 2014, with 27 countries participating at the outset. By the end of the first phase in 2018, membership had expanded to 67 countries, and as of 2022, the number of member countries reached 71. As of September 2022, the distribution of member countries includes 25 in the African region, 6 in the East Mediterranean region, 17 in the European region, 9 in the American region, 4 in the Southeast Asian region, and 10 in the Western Pacific region. During the first phase, the GHSA experienced a growth rate of approximately 150% in the number of participating countries. However, the second phase saw a more modest increase in membership. Looking ahead to the third phase, there is an expectation that non-member countries will join the GHSA, enhancing regional and multi-sectoral collaboration among countries with similar geographic and socio-cultural environments.

The primary objective of the Immunization Action Package is to enhance the immunization rates for vaccine-preventable diseases (VPDs). To attain a 95% immunization rate for VPDs, GHSA member countries must establish a multi-sectoral collaboration system. This system will facilitate policy-making and technical exchange, particularly for those countries with limited capacities in their NIPs. The Republic of Korea, in particular, is well-positioned to assist countries that require policy guidance on vaccine hesitancy and the management of routine immunization disruptions during the COVID-19 pandemic, as well as support with technologies such as vaccination registry systems.

The capacity for legal preparedness is a critical component in protecting against, detecting, and responding to infectious diseases among GHSA member countries. It is essential for achieving the objectives set forth in each Action Package. Effective collaboration between the Legal Preparedness Action Package and other Action Packages is necessary and requires robust communication. While the Republic of Korea has received commendation for its adept handling of COVID-19, which involved amending existing legislation and introducing new laws pertaining to infectious diseases following the MERS outbreak in 2015, there is a need for the country to assess the legal measures implemented and modified during the COVID-19 pandemic to better prepare for future outbreaks. Additionally, the Republic of Korea should endeavor to play a more proactive role in strengthening global health security through sustainable finance capacity.

With the emergence of Ebola, MERS, and Zika virus during the first phase of the GHSA, and COVID-19 in the second phase, member countries have become increasingly aware of the importance of health security. The GHSA Action Package requires the implementation of concrete measures to improve the ability to prevent, detect, and respond to emerging or re-emerging infectious diseases. Additionally, it is imperative for the GHSA to assess the performance and identify the challenges encountered during its second phase before embarking on the third phase, which spans from 2024 to 2028. To achieve this, member countries must consistently execute Action Packages and maintain robust financial and organizational capacities.

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Notes

Ethics Approval
Not applicable.

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

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

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

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Instruction for authors

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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 and correspondence, 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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Table of Contents

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  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.
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  3. Ministry of Employment and Labor. Statistics on occupational injuries and illnesses, 2008. Ministry of Employment and Labor; 2009.
- **Websites**
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- **Dissertation**
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