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Center of Excellence on Environmental Health and Toxicology

Proceedings of The 18th Annual Conference on Emerging Issues in Environmental Health

August 15th, 2026



at the Convention Center, Chulabhorn Research Institute, Bangkok, Thailand

Center of Excellence on Environmental Health and Toxicology
Annual Academic Conference 2026 on “Emerging Issues in Environmental Health”
Saturday, August 15, 2026
Chulabhorn Research Institute Convention Center, Bangkok

Saturday, August 15, 2026

08:30 - 09:00 Registration

09:00 - 09:10 Opening Address by *Professor Emeritus Khunying Mathuros Ruchirawat*

Director, Center of Excellence on Environmental Health and Toxicology

09:10 - 10:00 Keynote Lecture: “Emerging Challenges in Environmental Health Impacting Chronic Disease over the Next 25 Years” by *Professor John Groopman*

Chairman, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, USA

10:00 - 10:15 Group Photo and Coffee Break

10:15 - 11:15 Panel Discussion: “The current situation on health & environmental impacts of PFAS/PFOA” by

- **The PFAS/PFOA situation in Thailand** by *Dr. Phanit Ratsuk*

Director, Waste and Hazardous Substance Management Division, Pollution Control Department

- **Impacts of PFAS/PFOA on food and the environment** by

Dr. Teerapong Lertassavakorn, Mahidol University

- **Mechanisms of action and impacts of PFAS on non-communicable diseases (NCDs)** by

Dr. Phum Tachachartvanich, Chulabhorn Research Institute

Dr. Nutsira Vajeethaveesin, Chulabhorn Graduate Institute

Moderator: *Assistant Professor Puey Ounjai*, Mahidol University

11:15 - 12:00 Poster Presentations (poster presenters should be ready at their posters at 11.15 AM)

12:00 - 13:00 Lunch Break

13:00 - 14:00 Panel Discussion: “Heavy Metal Contamination of Natural Water Resources from Mining: A Case Study of the Kok River” by:

- *Dr. Wijarn Simachaya*, Director, Thailand Environment Institute and Expert, Thai National Research Council
- *Dr. Suthat Chotanaphan*, Deputy Director-General, Department of Disease Control
- *Dr. Chaiyo Juisiri*, Water Quality Management Specialist, Pollution Control Department
- *Ms. Pinyaphat Chunsuk*, Director of the Bureau of Food and Water Sanitation, Department of Health

Moderator: *Associate Professor Prayoon Fongsatitkul*

14:00 - 14:10 Introduction to the Hub of Talents Experts Database on Environmental Health by

Dr. Daam Settachan, Chulabhorn Research Institute

14:10 - 16:10 Student Workshop: “Research Leading to Policies and Strategies for Prevention, Control, and Remediation: The Source-Pathway-Receptor (SPR) Model Concept”

Group 1: Arsenic

Group 2: PFAS / PFOA

16:10 - 17:10 Student Workshop Presentations

17:10 - 17:30 Awards ceremony for poster presentations

17.30 Closing Comments by *Professor Emeritus Khunying Mathuros Ruchirawat*

Center of Excellence on Environmental Health and Toxicology (EHT)
Emerging Issues in Environmental Health
(2026)

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Prof. Dr. Khunying Mathuros Ruchirawat	Director, EHT
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Abbreviations

CGI	=	Chulabhorn Graduate Institute
CRI	=	Chulabhorn Research Institute
PH	=	Faculty of Public Health, Mahidol University
SCBI	=	Environmental Biology / Biology, Faculty of Science, Mahidol University
SCTX	=	Toxicology, Faculty of Science, Mahidol University
EHT	=	Center of Excellence on Environmental Health and Toxicology
MGGE	=	Molecular Genetics and Genetic Engineering, Mahidol University
PERDO	=	S&T Postgraduate Education and Research Development Office

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**Impacts of chemicals and
climate change on human
health and disease
development and factors
modulating such impacts**

APOPTOTIC EFFECTS OF TRICLOSAN EXPOSURE IN FOLLICULAR THYROID CARCINOMA FTC-133 CELLS

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Abstract:

Triclosan (TCS) is a widely used antimicrobial agent in personal care products that persists in the environment and raises concerns about potential effects on human health. This study aimed to investigate the apoptotic effects of TCS in FTC-133 cells. MTT cell viability assay was used to determine cytotoxicity, and apoptosis was evaluated using Annexin V-based analysis. Expression of apoptosis-associated proteins, including cleaved PARP, cleaved caspase-3, and DR5, was analyzed to elucidate the underlying mechanisms. In addition, Mitochondrial Outer Membrane Permeabilization (MOMP) was assessed by measuring mitochondrial membrane potential ($\Delta\Psi$) using the JC-1 assay to evaluate mitochondrial involvement. The results showed that TCS treatment significantly reduced cell viability and increased Annexin V-positive cells at 24 h. These results indicate that TCS can cause apoptosis in FTC-133 cells. Moreover, TCS induced caspase activation, as evidenced by increased cleaved caspase-3 and cleaved PARP at 3 and 24 h after TCS treatment, suggesting that caspase activation participates in TCS-induced apoptosis. Furthermore, $\Delta\Psi$ measurements revealed a significant reduction following TCS treatment at 1 and 3 h. Additionally, an increase in DR5 expression was observed at 24 h after TCS treatment, suggesting that TCS may also induce extrinsic apoptosis. Together, the findings of the present study suggest that TCS induces apoptosis in FTC-133 cells, associated with early mitochondrial dysfunction and DR5-related signaling.

Keywords: Triclosan, Apoptosis, DR5 signaling, Mitochondrial dysfunction, Thyroid, Thyrocytes

Introduction:

Triclosan (TCS) is a widely used antimicrobial agent found in many personal care products (1). Due to its extensive use and environmental persistence, concerns have been raised regarding its potential health effects, including disruption of thyroid hormone regulation (2). However, information regarding the effects of TCS on thyroid follicular cells (thyrocytes) remains limited. Therefore, this study aimed to investigate the apoptotic effect of TCS on thyrocytes using the follicular thyroid carcinoma FTC-133 cell line.

Methodology:

Cell culture: FTC-133 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)/Ham's F-12 (1:1) supplemented with 10% FBS and 1% Penicillin-Streptomycin. Cells were treated with varying concentrations of TCS and different treatment times.

MTT cell viability Assay: FTC-133 cells were treated with 0 - 80 μ g/ml TCS for 24 hours. After treatment, MTT solution was added and incubated for 4 hours at 37°C, followed by DMSO addition to dissolve MTT insoluble crystal. Cell viability was measured using a microplate reader at 570 nm.

Annexin V: FTC-133 cells were treated with 0, 5, 10, and 20 $\mu\text{g/ml}$ TCS for 3 or 24 hours. Cells were stained with FITC Annexin V and PI, then analyzed by flow cytometry to detect apoptotic cells.

Western blot: FTC-133 cells were treated with 0, 5, 10, and 20 $\mu\text{g/ml}$ TCS for 3 or 24 hours. Whole-cell lysates were prepared, separated by SDS-PAGE, and transferred to nitrocellulose membranes. After antibody incubation, protein expression was detected using ECL and visualized with a Chemidoc imaging system.

JC-1 Assay: FTC-133 cells were treated with 0, 10, and 20 $\mu\text{g/ml}$ TCS for 1 or 3 hours. Cells were stained with JC-1 dye and analyzed by flow cytometry to assess mitochondrial membrane potential ($\Delta\Psi\text{M}$).

Results and discussion:

TCS reduces cell viability

The results showed that TCS decreased FTC-133 cell viability in a dose-dependent manner after 24 hours of treatment. Significant cytotoxicity was observed at concentrations $\geq 10 \mu\text{g/ml}$, while 80 $\mu\text{g/ml}$ showed the strongest effect (Fig. 1).

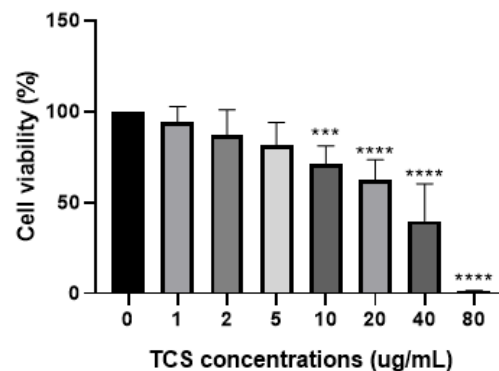


Figure 1 Concentration-response pattern of TCS-induced cytotoxicity in FTC-133 cells following 24 hours of treatment.

TCS induces cell death through apoptosis pathway

TCS did not significantly induce apoptosis after 3 hours of treatment (Fig. 2A). However, 24 hours of treatment significantly increased Annexin V-positive cells at 10 and 20 $\mu\text{g/ml}$, indicating apoptosis induction in FTC-133 cells (Fig. 2B).

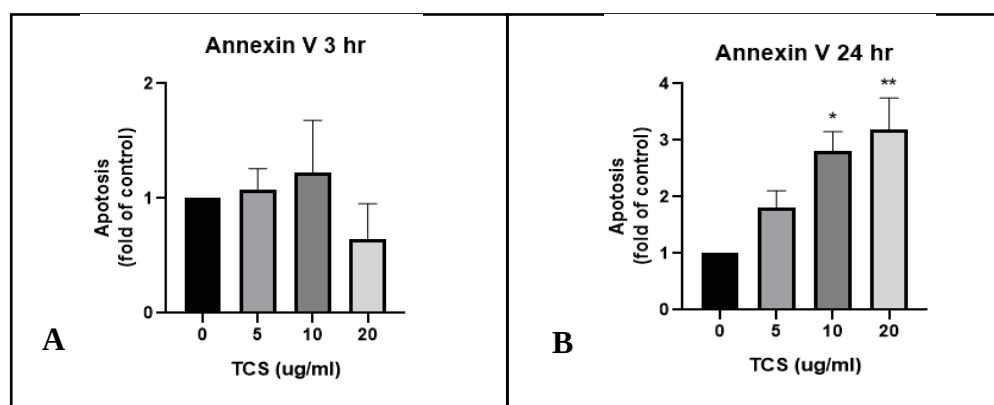


Figure 2 Apoptosis induction by TCS. (A, B) Percentage of apoptosis quantified by flow cytometry from 3 and 24 hours of TCS treatment.

TCS activates the caspase signaling pathway characterized by cleaved PARP and cleaved caspase-3

Caspase activation is the molecular hallmark of apoptosis (3). TCS increased cleaved PARP (Fig. 3A) and cleaved caspase-3 (Fig. 3B) expression, particularly after 24 hours of treatment at higher concentrations, suggesting activation of caspase-dependent apoptosis.

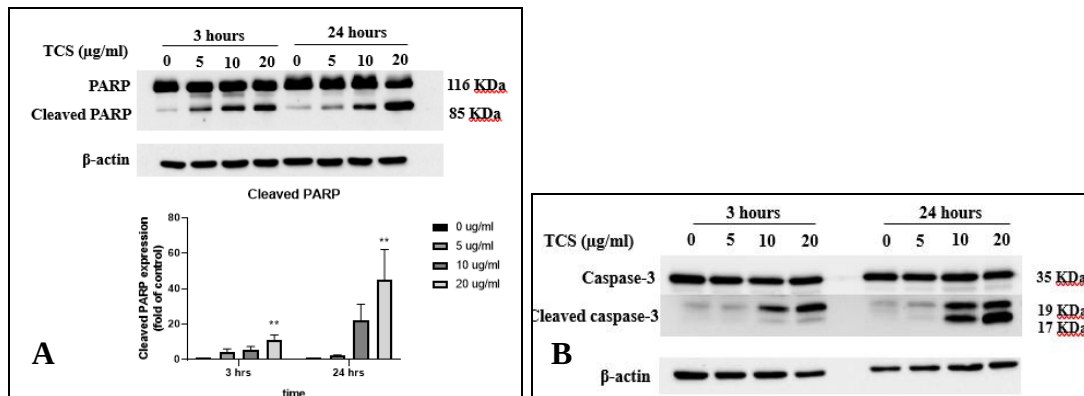


Figure 3 Cleaved PARP and cleaved caspase-3 levels after TCS treatment. (A) Representative blots and quantitative protein levels of cleaved PARP were normalized with β-actin and expressed as fold change relative to the control. (B) Representative blots of caspase-3, cleaved caspase-3, and β-actin.

TCS effects on mitochondrial membrane potential ($\Delta\Psi_M$) in FTC-133 cells

Changes in $\Delta\Psi_M$ are associated with the intrinsic apoptosis (4). TCS treatment reduced $\Delta\Psi_M$ at both time points, as indicated by the shift from red to green fluorescence compared to the control. At 1 hour, 10 and 20 μg/ml initiated a shift from red to green fluorescence, suggesting early mitochondrial depolarization (Fig. 4A). This effect became sustained at 3 hours, particularly at 20 μg/ml (Fig. 4B). These findings indicate that TCS rapidly disrupts mitochondrial membrane potential, suggesting early mitochondrial dysfunction that may contribute to activation of the intrinsic apoptotic pathway.

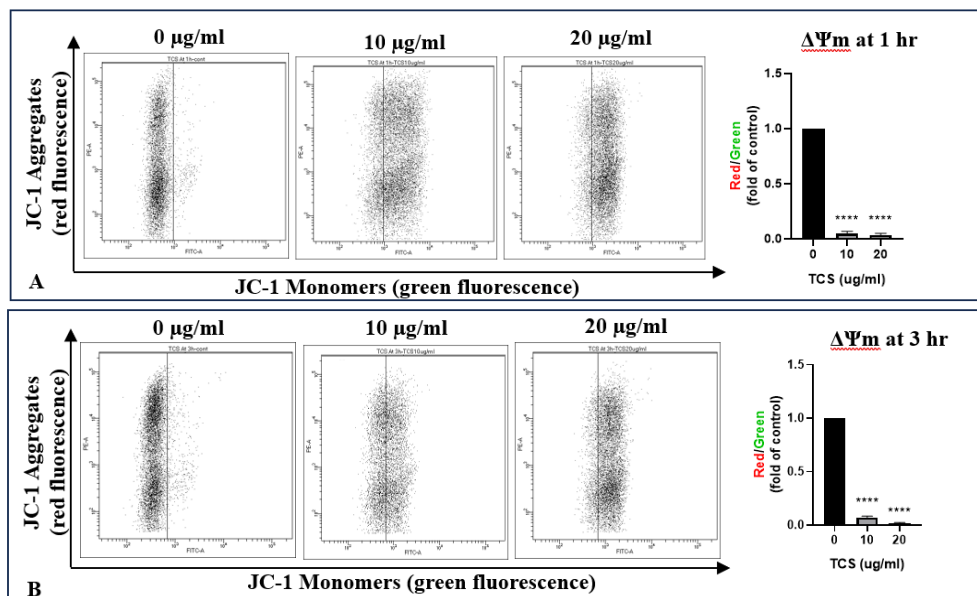


Figure 4 Effect of TCS on $\Delta\Psi_M$. (A, B) Representative flow cytometry dot plots showing JC-1 aggregates (red fluorescence) and JC-1 monomers (green fluorescence), and quantification of Red/Green fluorescence at 1 (A) and 3 hours (B) of TCS treatment, respectively, and presented as a percentage of the control.

TCS alters a protein involved in the extrinsic apoptosis pathway

DR5 is a key mediator of the extrinsic apoptotic pathway (5). DR5 expression was examined by Western blotting. TCS treatment at 3 hours showed no significant change. However, at 24 hours, DR5 expression was markedly increased, with a significant increase observed at 20 $\mu\text{g/ml}$ (Fig. 5). These findings suggest that TCS induces DR5 expression, potentially activating the extrinsic apoptosis pathway.

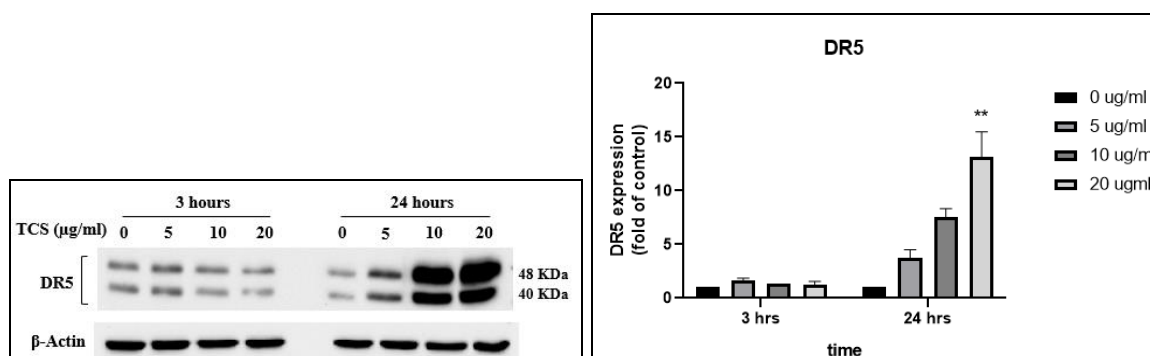


Figure 5 Effect of TCS on DR5 expression in FTC-133 cells. Representative blot of DR5 and quantification of DR5 protein expression normalized to β -actin and expressed as fold change relative to the control

Conclusion:

Overall, these findings indicate that TCS induces apoptosis associated with early mitochondrial dysfunction and DR5-related signaling in FTC-133 cells.

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ASSESSMENT OF GREENHOUSE GAS EMISSION REDUCTIONS FROM BANGKOK METROPOLITAN ADMINISTRATION DISTRICT OFFICES: A CASE STUDY OF SATHON DISTRICT OFFICE

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Abstract:

Global warming and climate change have become critical global challenges driven primarily by greenhouse gas (GHG) emissions from human activities. Public sector organizations play an important role in reducing emissions through improved energy efficiency and effective carbon management strategies. This study aimed to assess the organizational carbon footprint (CFP) of the Sathon District Office, Bangkok, Thailand, identify major sources of greenhouse gas emissions, and propose appropriate mitigation measures to support sustainable organizational management. Secondary data on greenhouse gas emissions generated from the operational activities of the Sathon District Office were collected for the reporting period from October 2023 to September 2024. The organizational greenhouse gas inventory was developed by classifying emissions into Scope 1, Scope 2, and Scope 3 in accordance with the Carbon Footprint for Organization (CFO) Guidelines issued by the Thailand Greenhouse Gas Management Organization (TGO), consistent with ISO 14064-1:2018, the Greenhouse Gas Protocol, and ISO/TR 14069:2013. The results showed that the total organizational greenhouse gas emissions were 43,568.66 tCO_{2e}. Scope 3 emissions were the dominant contributor, accounting for the majority of total emissions (41,970.58 tCO_{2e}), followed by Scope 2 emissions (826.32 tCO_{2e}) and Scope 1 emissions (771.76 tCO_{2e}).

The findings indicate that greenhouse gas mitigation efforts should prioritize reducing Scope 3 emissions, particularly those associated with municipal waste management, while continuing to improve energy efficiency and fuel management in operational activities. This study provides baseline greenhouse gas emission data for the Sathon District Office and offers practical guidance for local government organizations in developing carbon footprint inventories and implementing effective mitigation strategies in support of long-term carbon neutrality and national climate change goals.

Keywords: Organizational Carbon Footprint, Greenhouse Gas Inventory, Greenhouse Gas Emissions, Public Sector, Greenhouse Gas Mitigation

Introduction:

Global warming and climate change have emerged as critical global challenges, adversely affecting economic development and the quality of life of populations worldwide. These challenges are primarily driven by anthropogenic activities, particularly economic production and consumption, which continuously release greenhouse gases (GHGs) into the atmosphere. Recent studies have highlighted the variability of greenhouse gas emission profiles among public sector organizations. For example, a study on organizational carbon footprint assessment and engineering approaches for reducing greenhouse gas emissions at

the Pollution Control Department found that Scope 2 emissions, representing indirect greenhouse gas emissions from electricity consumption, were the largest source of emissions ¹. Similarly, an organizational carbon footprint assessment conducted at the Department of Energy Human Resource Development identified purchased electricity (Scope 2) as the predominant contributor to total greenhouse gas emissions ². In contrast, a carbon footprint assessment of a local government organization, using the Bang Khae District Office as a case study, found that Scope 3 emissions were the largest emission source, with the majority resulting from employees' commuting between their residences and the workplace ³. Additional studies have also reported variations in greenhouse gas emission patterns across public organizations, depending on their operational characteristics and organizational contexts.

These findings suggest that the magnitude and distribution of greenhouse gas emissions differ considerably among government agencies, reflecting differences in operational activities, energy consumption patterns, and organizational environments. Therefore, conducting organization-specific greenhouse gas inventories is essential for identifying major emission sources and developing effective emission reduction strategies tailored to the operational context of each organization.

Materials and Methods:

This study was conducted in Sathorn District, Bangkok, Thailand. Secondary data on greenhouse gas (GHG) emission sources generated from the operational activities of the Sathorn District Office were collected and analyzed for the reporting period from October 2023 to September 2024. The organizational greenhouse gas inventory was developed in accordance with the Carbon Footprint for Organization (CFO) Guidelines issued by the Thailand Greenhouse Gas Management Organization (Public Organization) (TGO).

Greenhouse gas emissions were classified into three scopes. Scope 1 included direct emissions from stationary and mobile fuel combustion (diesel fuel and gasoline) and on-site wastewater treatment using septic tank systems. Scope 2 includes indirect emissions associated with purchased electricity consumption. Scope 3 encompasses other indirect emissions from the following categories: Category 3.1: Consumption of office resources, Category 3.3: Fuel and energy-related activities, and Category 3.5: Management of municipal solid waste and fuel used in associated activities. These categories are defined in the TGO guidelines.

Results and Discussion:

The greenhouse gas inventory of the Sathorn District Office was assessed for the reporting period from October 2023 to September 2024. The results showed that greenhouse gas emissions amounted to 771.76 tCO₂e from Scope 1 (direct greenhouse gas emissions), 826.32 tCO₂e from Scope 2 (indirect greenhouse gas emissions associated with purchased electricity consumption), and 41,970.58 tCO₂e from Scope 3 (other indirect greenhouse gas emissions).

The results indicate that Scope 3 emissions were the dominant contributor to the organizational carbon footprint, substantially exceeding emissions from Scope 1 and Scope 2. A major proportion of these emissions originated from Category 3.5, which included fuel consumption by municipal solid waste collection vehicles and municipal solid waste management throughout the Sathorn District. Additional Scope 3 emission sources included A4 paper consumption, municipal water consumption, and office waste management. The high contribution of Category 3.5 reflects the district office's responsibility for municipal waste collection and transportation, which requires continuous vehicle operation and fuel consumption. The detailed greenhouse gas emission sources included in each scope are presented in Table 1.

This finding differs from several previous studies of Thai public sector organizations, where Scope 2 emissions from purchased electricity consumption were identified as the primary source of greenhouse gas emissions. The difference may be attributed to variations in organizational responsibilities, operational characteristics, and emission sources, particularly due to the inclusion of municipal solid waste collection activities within the Sathorn District Office's organizational boundary.

The findings suggest that greenhouse gas mitigation strategies should extend beyond improving energy efficiency within office operations. Priority should be given to reducing emissions from municipal waste management by optimizing waste collection routes, improving vehicle fuel efficiency, adopting low-emission or alternative-fuel waste collection vehicles where feasible, and promoting waste reduction and source segregation to minimize the volume of waste requiring collection and disposal. These measures, together with improvements in electricity efficiency and fuel management for other operational activities, would contribute to reducing the organization's overall carbon footprint.

Table 1 Summary of total GHG emissions by scope.

Scope	Category	GHG emission (tCO ₂ eq/year)	% of Scope total
Scope 1	Stationary combustion	49.87	6.46
	Mobile combustion	705.69	91.44
	Fugitive emissions	16.20	2.1
	Total	771.76	100%
Scope 2	Purchased electricity	826.32	100%
Scope 3	Category 3.1: Purchased goods and services	29.40	0.07
	Category 3.3: Fuel- and energy-related activities	231.28	0.55
	Category 3.5: Waste generated and fuel related activities in operations	41,709.90	99.38
	Total	41,970.58	100%

The assessment results were subsequently used to develop policy recommendations and identify appropriate energy conservation and greenhouse gas mitigation measures for the Sathorn District Office.

$$\text{GHG Emissions (tCO}_2\text{e)} = \sum (\text{Activity Data} \times \text{Emission Factor}) \quad (1)$$

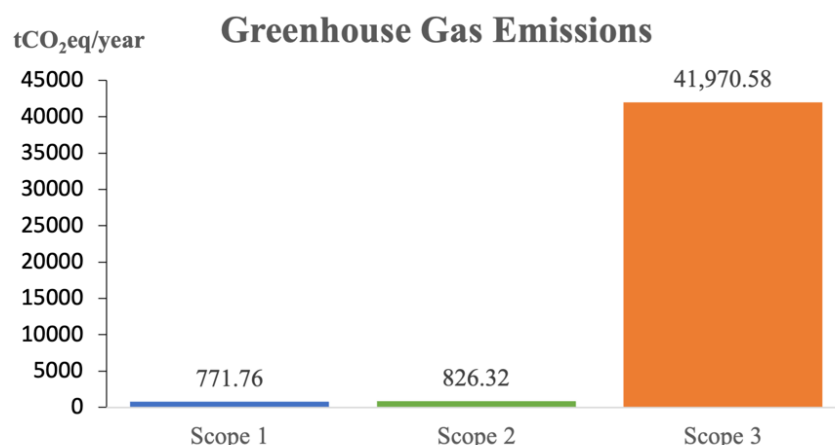


Figure 1 Greenhouse gas emissions from Scope 1, Scope 2, and Scope 3 activities.

Conclusion:

This study assessed the organizational carbon footprint of the Sathorn District Office for the reporting period from October 2023 to September 2024 in accordance with the Carbon Footprint for Organization (CFO) Guidelines of the Thailand Greenhouse Gas Management Organization (TGO). The findings indicate that Scope 3 emissions constituted the largest share of the organization's greenhouse gas emissions, primarily due to municipal solid waste management activities, particularly fuel consumption for waste collection and transportation. In contrast, emissions from Scope 2 (purchased electricity consumption) and Scope 1 (direct operational activities) accounted for smaller proportions of the total organizational carbon footprint.

The results highlight the importance of prioritizing greenhouse gas mitigation measures for municipal waste management. Recommended measures include optimizing waste collection routes, improving the fuel efficiency of waste collection vehicles, promoting waste reduction and source segregation, and gradually adopting low-emission or alternative-fuel vehicles where technically and economically feasible. In addition, Scope 1 emissions could be reduced through improved fuel management, regular maintenance of vehicles and equipment, optimization of fuel consumption, the transition from conventional diesel to B7 biodiesel where appropriate, and the gradual replacement of conventional vehicles with electric vehicles (EVs) as infrastructure and operational conditions permit. Scope 2 emissions can be further reduced by enhancing electricity efficiency. This can be achieved by installing energy-efficient lighting and equipment, optimizing air-conditioning systems, promoting energy-saving practices among staff, and installing solar photovoltaic (PV) systems to generate renewable electricity for office operations when possible. Continuous monitoring of greenhouse gas emissions and periodic updates of the organizational carbon footprint inventory are recommended to support evidence-based decision-making and evaluate the effectiveness of mitigation measures over time.

This study provides baseline greenhouse gas emission data for the Sathorn District Office and offers practical information for developing greenhouse gas reduction policies and organizational carbon footprint inventories in local government organizations. Future research should extend the assessment to additional district offices or municipalities to enable comparative analyses, identify best practices for greenhouse gas mitigation, and support Thailand's long-term carbon neutrality and climate change mitigation goals.

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CHARACTERIZATION OF A NOVEL MECHANISM ASSOCIATED WITH REDUCED β -LACTAM ANTIBIOTICS SUSCEPTIBILITY IN *Stenotrophomonas maltophilia*

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Abstract:

Stenotrophomonas maltophilia is an opportunistic multidrug-resistant pathogen that poses a significant therapeutic challenge because of its intrinsic resistance to multiple classes of antimicrobial agents. Although ceftazidime/avibactam (CZA) has demonstrated activity against multidrug-resistant Gram-negative bacteria, reduced susceptibility to this agent has increasingly been reported, and the underlying resistance mechanisms in *S. maltophilia* remain poorly understood. This study aimed to characterize the antimicrobial susceptibility profile of a CZA-resistant mutant and investigate the contribution of a TonB-dependent energy transduction (TBDET)-associated gene to the observed phenotype.

A spontaneous CZA-resistant mutant, designated KCA4, was generated from *S. maltophilia* K279a by serial exposure to increasing concentrations of CZA using the broth disk elution (BDE) method. Antibiotic susceptibility was evaluated by the Kirby-Bauer disk diffusion assay, genomic alterations were identified by whole-genome sequencing (WGS), and functional validation was performed through complementation with the TBDET-associated gene. Compared with the parental strain, KCA4 exhibited reduced susceptibility to CZA and altered susceptibility to multiple antibiotic classes, particularly β -lactam antibiotics. WGS identified a frameshift mutation in the TBDET-associated gene. Complementation with the TBDET-associated gene restored antibiotic susceptibility to a level comparable with that of the parental strain, demonstrating that disruption of this gene contributes to the reduced susceptibility observed in KCA4.

These findings demonstrate that loss of TBDET function contributes to reduced susceptibility to CZA and several additional antimicrobial agents in *S. maltophilia*. This study identifies the TonB-dependent transport system as a potential determinant of antimicrobial susceptibility and provides new insight into a non-classical mechanism of antimicrobial resistance in this clinically important pathogen.

Keywords: *Stenotrophomonas maltophilia*, Multidrug resistance (MDR), β -lactam resistance, Ceftazidime/avibactam (CZA), TonB-dependent energy transduction (TBDET)

Introduction:

Antimicrobial resistance (AMR) in Gram-negative bacteria is a major global health concern because it limits the effectiveness of available antimicrobial therapies (1). *Stenotrophomonas maltophilia* is an opportunistic multidrug-resistant pathogen that is intrinsically resistant to multiple antibiotic classes, making infections difficult to treat (2). Ceftazidime/avibactam (CZA), a β -lactam/ β -lactamase inhibitor combination, has shown activity against multidrug-resistant Gram-negative bacteria (3). However, reduced susceptibility to CZA has

increasingly been reported and is commonly associated with metallo- β -lactamase production or acquired β -lactamases (4). Although resistance is often attributed to metallo- β -lactamase production, this mechanism alone does not fully explain the reduced susceptibility observed in *S. maltophilia*, suggesting the involvement of additional non-classical resistance mechanisms. Therefore, this study generated and characterized a spontaneous CZA-resistant mutant of *S. maltophilia* K279a using antimicrobial susceptibility testing, whole-genome sequencing (WGS), and genetic complementation to investigate the role of a TonB-dependent energy transduction (TBDET)-associated gene in reduced CZA susceptibility.

Methodology:

Mutant selection

The wild-type *S. maltophilia* K279a was serially exposed to increasing concentrations of CZA using the broth disk elution (BDE) method. A spontaneous CZA-resistant mutant (KCA4) was isolated from a colony growing within the inhibition zone of CZA E-test.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method. A bacterial suspension adjusted to OD₆₀₀ = 0.132 was swabbed onto Mueller-Hinton agar, followed by the application of antibiotic disks. After 20-24 hours of incubation, inhibition zones were measured.

Gene mutation identification

Genomic DNA from KCA4 was subjected to WGS using the Illumina MiSeq platform. Sequence reads were quality-filtered and aligned to the reference genome of *S. maltophilia* K279a (accession no: NC_010943). Genetic variants, including single nucleotide polymorphisms (SNPs) and insertion/deletion mutations, were identified to determine candidate mutations associated with the resistant phenotype.

Functional validation

To determine the contribution of TBDET-associated gene to the reduced susceptibility phenotype, the wild-type of this gene was cloned into pBBRCM vector and introduced into KCA4 by electroporation. Antibiotic susceptibility of the complemented strain was evaluated by disk diffusion assay and compared with those of the parental and mutant strains.

Results and Discussion:

Changes in antimicrobial susceptibility following CZA exposure

Serial exposure of *S. maltophilia* K279a to increasing concentrations of CZA using the BDE method resulted in the isolation of a spontaneous mutant, designated KCA4. Compared with the parental strain, KCA4 exhibited reduced susceptibility to CZA, as demonstrated by a smaller inhibition zone. In addition, KCA4 displayed reduced susceptibility to several β -lactam antibiotics and altered susceptibility to other non- β -lactam agents (Figure 1). These findings suggest that adaptation to CZA affects susceptibility to multiple antibiotic classes and may involve mechanisms beyond classical β -lactamase-mediated resistance.

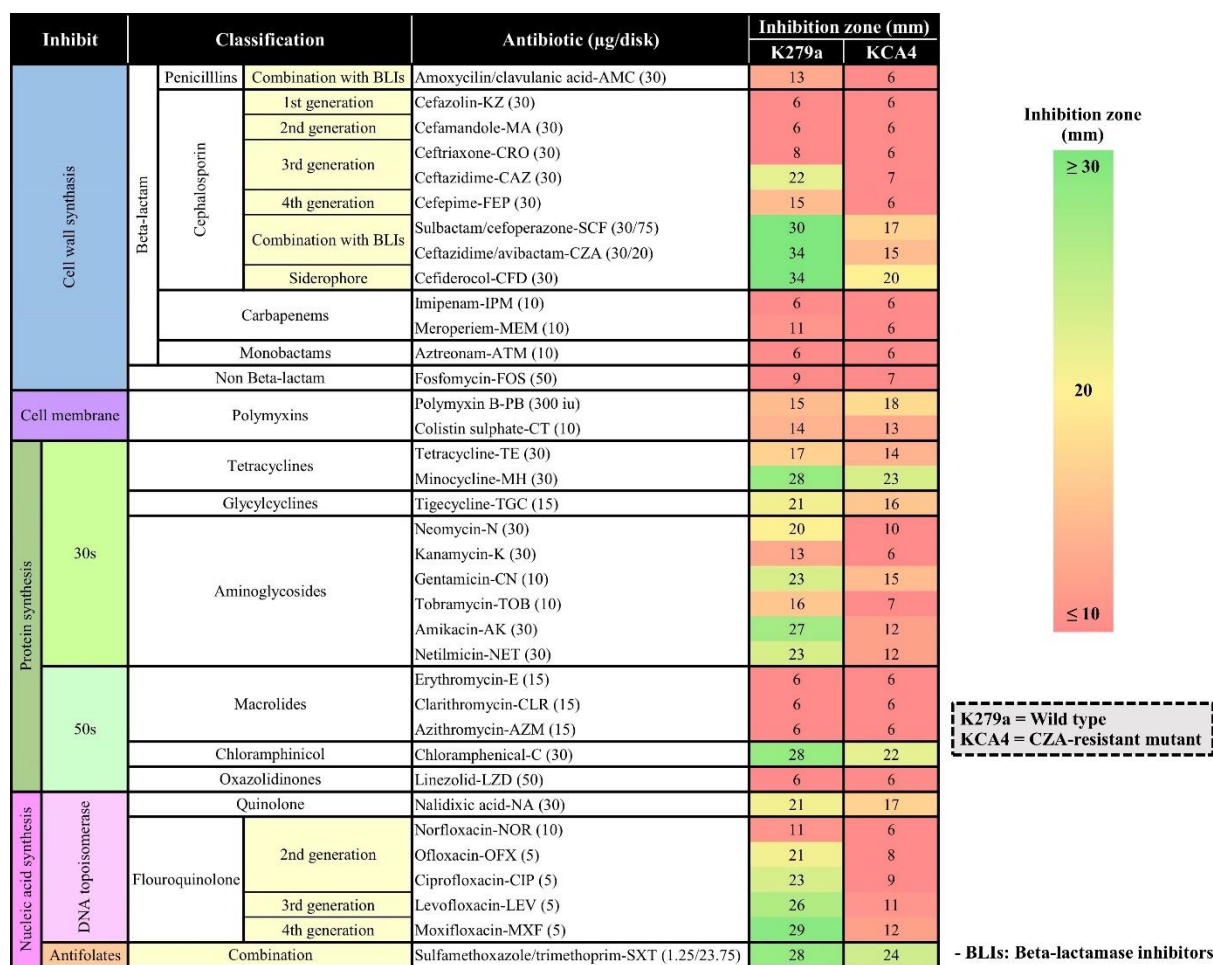


Figure 1 Antimicrobial susceptibility profiles of the CZA-resistant mutant compared with the parental strain K279a. Heatmap showing inhibition zone diameters (mm) determined by the Kirby-Bauer disk diffusion assay against antibiotics from different antibiotic classes. Colors indicate the size of inhibition zone diameters.

Identification of mutations in the CZA-resistant mutant

WGS of KCA4 identified a frameshift mutation in the TBDET-associated gene. This gene is located within the gene cluster of TonB-dependent transport system (Figure 2), which encodes the TonB-dependent energy transduction complex responsible for powering nutrient transport across the outer membrane (5). Disruption of this gene impair TonB-dependent transport, which may reduce antibiotic uptake and contribute to decreased CZA susceptibility through a mechanism independent of classical β -lactamase-mediated resistance (6).

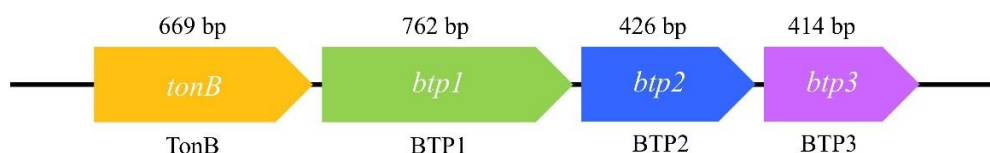


Figure 2 Genetic organization of the TonB-dependent transport system gene cluster in *S. maltophilia* K279a. The identified frameshift mutation is located in the *btp3* gene within the conserved *tonB-btp1-btp2-btp3* gene cluster, which encodes components of the TonB-dependent transport system.

Restoration of antibiotic susceptibility after complementation

To determine whether TBDET-associated gene contributed to the reduced susceptibility phenotype, this gene was introduced into KCA4 by plasmid complementation. The complemented strain, KCA4/pBBRCM*mbtp3*, exhibited increased inhibition zone diameters against tested antibiotic agents, approaching those of the parental strain, K279a/pBBRCM (Figure 3). Restoration of antimicrobial susceptibility following complementation demonstrates that disruption of this gene contributed to the reduced susceptibility phenotype of KCA4, despite the presence of additional genomic mutations.

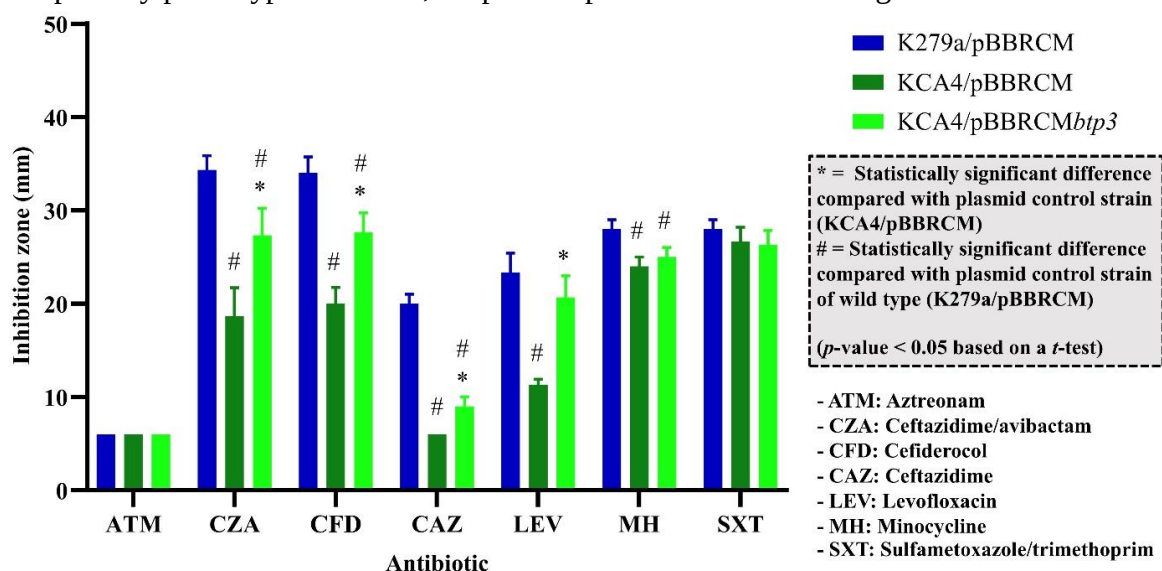


Figure 3 Restoration of antibiotic susceptibility following complementation of the CZA-resistant mutant. Complementation with the TBDET-associated gene increased inhibition zone diameters compared with the mutant strain, KCA4/pBBRCM. Data are presented as mean $\pm$ SD from three independent experiments.

Conclusion:

Serial exposure of *S. maltophilia* K279a to CZA generated a mutant with reduced susceptibility to CZA and multiple antibiotic agents. WGS identified a frameshift mutation in a TBDET-associated gene, and complementation restored antibiotic susceptibility. These findings identify the TonB-dependent transport system as a potential determinant of antimicrobial susceptibility.

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EFFECTS OF DIISONONYL CYCLOHEXANE -1,2- DICARBOXYLATE (DINCH) ON HUMAN HEMATOPOIETIC DEVELOPMENT

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Abstract:

Among the various types of plasticizers available, phthalates have been the most extensively utilized. Dibutyl phthalate (DBP) and Di(2-ethylhexyl) phthalate (DEHP) are among the most well-recognized phthalates and have been identified in numerous studies as hazardous substances due to their endocrine-disrupting properties and associated risks to human health. In response to these concerns, there has been a shift toward using non-phthalate alternative plasticizers, such as Diisononyl cyclohexane-1,2-dicarboxylate (DINCH). Following its approval by the European Food Safety Authority in 2007, DINCH is increasingly used as a substitute for phthalates in several consumer products, medical devices, and personal care items, leading to widespread human exposure. The detection of metabolites of these plasticizers in maternal and cord blood confirms prenatal exposure during critical developmental windows. However, toxicity data of DINCH remain limited, particularly regarding its effects on stem cell biology and hematopoietic differentiation. This study aimed to investigate the effects of exposure to selected plasticizers on pluripotency maintenance and hematopoietic differentiation using an *in vitro* differentiation model. Human induced pluripotent stem cells (iPSCs) were exposed to DBP, DEHP, and DINCH (0.1-10 μ M) during pluripotency maintenance or exposed to DEHP and DINCH (0.01-10 μ M) during hematopoietic differentiation. The expression of pluripotency-associated genes was assessed on Day3 by quantitative real-time PCR, while development of hematopoietic stem and progenitors (HSPCs) was evaluated by flow cytometric analysis of CD34, CD43, and CD45 expression. Initial screening showed that DBP and DINCH did not significantly affect the expression of *NANOG*, *SOX2*, or *OCT3/4* transcripts. In contrast, treatment with 10 μ M DEHP significantly reduced *SOX2* expression. Therefore, DEHP and DINCH were selected for further investigation during hematopoietic differentiation. The results showed that both DINCH and DEHP significantly reduced the number of CD34⁺ cells. However, only DEHP significantly increased CD34⁺CD43⁺CD45⁻ cells while decreased CD34⁺CD43⁺CD45⁺ cells. Collectively, these findings demonstrate that DEHP exerts more pronounced effects than DINCH on hematopoietic progenitor development and maturation highlighting the utility of human iPSC-based models for evaluating the developmental toxicity of emerging plasticizers.

Keywords: Dibutyl phthalate, Di(2-ethylhexyl) phthalate, Diisononyl cyclohexane-1,2-dicarboxylate, Human inducible pluripotent stem cells, Hematopoietic differentiation.

Introduction:

Plasticizers refer to chemicals that are added into materials to enhance their flexibility and durability, which making them better suited for specific applications. Among the various class of plasticizers, phthalates including dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) have been widely used in the production and processing of flexible

polyvinyl chloride (PVC) plastics (1). Phthalates are not chemically bound to plastics, so they leach easily into the environment (2). Human exposure occurs through ingestion of contaminated food and water, inhalation of indoor air and dust, and dermal contact with consumer products (3, 4). Toxicological studies have demonstrated that both DBP and DEHP are not only disrupt endocrine system but also affect several organs including immune system. Due to various health concerns associated with phthalates, alternative plasticizers such as Diisononyl cyclohexane-1,2-dicarboxylate (DINCH) have been increasingly adopted. Following its approved by the European Food Safety Authority in 2007 (5), DINCH has increasingly replaced phthalates in food-contact materials, medical devices, children's products, and personal care items. Consequently, human exposure to DINCH has risen substantially. Biomonitoring studies have detected metabolites of DBP, DEHP, and DINCH in amniotic fluid, breast milk, and cord blood, indicating that exposure occurs during critical stages of prenatal development (7-9). Although DINCH has generally been considered a safer alternative, emerging evidence indicates that it may induce endocrine-related effects comparable to those reported for conventional phthalates (6). Nevertheless, its potential developmental toxicity, particularly its effects on early hematopoietic development, remains poorly understood.

Developmental exposure to environmental contaminants is of particular concern because early embryonic and fetal stages are highly sensitive to chemical perturbation. Hematopoietic stem and progenitor cells (HSPCs) emerge during embryogenesis and give rise to all blood and immune cell lineages throughout life. Disruption of hematopoietic development during this period may alter progenitor cell composition and functional capacity, thereby affecting lifelong hematopoietic and immune homeostasis. Therefore, evaluating the effects of environmental chemicals on embryonic hematopoiesis is essential for understanding their potential developmental immunotoxicity.

Human induced pluripotent stem cells (iPSCs) provide a physiologically relevant in vitro model for investigating the effects of environmental chemicals on early developmental processes, including hematopoietic differentiation. Therefore, this study aimed to investigate the effects of DBP, DEHP and DINCH on pluripotency maintenance and hematopoietic progenitor development using a human iPSC-based hematopoietic differentiation model.

Objectives:

1. To investigate the effects of selected plasticizers on the expression of pluripotency-associated genes in human iPSCs.
2. To evaluate the effects of selected plasticizers on hematopoietic differentiation potential of human iPSCs.

Methodology:

1. Cell culture and treatment

Human iPSCs were obtained from the American Type Culture Collection (ATCC, USA) and maintained in mTeSR™ PLUS medium (STEMCELL™ TECHNOLOGIES). Differentiation of human iPSCs into HSPCs were induced using hematopoietic differentiation medium (STEMdiff™ Hematopoietic Kit, STEMCELL™ TECHNOLOGIES) according to the manufacturer's instructions. Cells were seeded onto Vitronectin-coated 12-well plates as small aggregates (approximately 50-100 µm in diameter) at a density of 20-40 aggregates per well and incubated at 37 °C with 5% of CO₂. Cells were then treated with plasticizers at various concentrations (0.01-10 µM) on Days 0, 2, 3, 5, 7, and 10 of the differentiation.

2. Flow cytometric Analysis

To evaluate cell viability and hematopoietic differentiation, floating cells were collected on Day 12 and analyzed by flow cytometry. Cell viability was assessed using LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific, USA). Surface expression of hematopoietic markers was assessed using FITC anti-human CD34 antibody, clone 581 (Catalog #343504, BioLegend, USA), APC anti-human CD43 antibody, clone CD43-10G7 (Catalog #343206, BioLegend), and PE anti-human CD45 antibody, clone HI30 (Catalog #304008, BioLegend) according to the manufacturer's instruction. Samples were analyzed using a BD FACSDiscover™ S8 Cell Sorter (BD Biosciences, USA).

3. Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was isolated using the RNeasy® Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. RNA concentration and purity were determined using a NANODROP ONE spectrophotometer (Thermo Fisher Scientific). Total RNA was reverse transcribed into complementary DNA (cDNA) using the qScript™ cDNA Synthesis Kit (Quantabio, USA) according to the manufacturer's protocol. Reverse transcription was performed using a PCR Mastercycler (Model: nexus GSX1; Eppendorf, USA). The expression levels of genes associated with stem cell pluripotency (*NANOG*, *SOX2*, and *OCT3/4*) were evaluated. Ribosomal Protein L13A (*RPL13A*) was served as the reference (housekeeping) gene for normalization. qPCR was performed using Toyobo SYBR Green Master Mix (Toyobo, Japan) on a CFX Opus 96 Real-Time PCR System (Bio-Rad, Inc., USA). Relative mRNA expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to *RPL13A* transcript expression.

4. Statistical Analysis

The statistically analysis was performed using the GraphPad Prism software (version 11.0.2; GraphPad, USA). Results are expressed as the mean $\pm$ standard error of the mean (SEM). Two-way analysis of variance (ANOVA) with a Dunnett's test was used to determine the difference between the treatment groups relative to the vehicle control. p -value < 0.05 indicates a statistically significant difference.

Results and Discussion:

1. Effects of exposure to selected plasticizers on the expression of pluripotency-associated genes in human iPSCs

Human iPSCs were treated with DBP, DEHP, and DINCH at different concentrations (0.1, 1, and 10 μ M). The expression levels of key pluripotency-associated genes, including *NANOG*, *SOX2*, and *OCT3/4*, were determined by RT-qPCR. Treatment with DBP or DINCH did not significantly affect the expression of the investigated genes. Similarly, DEHP treatment did not significantly affect the expression of *NANOG* or *OCT3/4*. However, a significant decrease in *SOX2* expression was observed in the 10 μ M DEHP-treated group compared with the vehicle control group. The cooperative expression of *SOX2*, *OCT3/4*, and *NANOG* constitute a core transcriptional network that maintains pluripotency in human pluripotent stem cells. Therefore, the selective reduction in *SOX2* expression following DEHP exposure may reflect an early perturbation of pluripotency-associated regulatory pathways rather than a complete loss of pluripotency. Further studies examining protein levels are required to determine whether DEHP exposure affects the maintenance of the pluripotent state in human iPSCs.

2. Effects of exposure to selected plasticizers on hematopoietic progenitor development from human iPSCs

Based on the initial screening experiment, subsequent investigations focused on DEHP and DINCH, which represent a conventional phthalate plasticizer and a widely used alternative plasticizer, respectively. To investigate their effects on hematopoietic progenitor development, human iPSCs were cultured in hematopoietic differentiation medium in the presence or absence of DEHP or DINCH (0.01, 0.1, 1, and 10 μ M). The generation of HSPC-like populations was assessed by flow cytometric analysis of hematopoietic marker expression (CD34, CD43, and CD45). Treatment with 10 μ M DEHP significantly reduced the number of CD34⁺ and CD34⁺CD43⁺CD45⁺ cells, while increased the number of CD34⁺CD43⁺CD45⁻ cells compared with the vehicle control group. Since expression of CD45 is associated with progression toward a more mature hematopoietic phenotype (10), these findings suggest that DEHP exposure may alter the developmental progression of HSPCs during differentiation. DINCH treatment significantly reduced the number of CD34⁺ cells at 1 and 10 μ M; however, no significant change was observed in the CD34⁺CD43⁺CD45⁻ nor CD34⁺CD43⁺CD45⁺ populations. These findings suggest that DEHP, but not DINCH, significantly affected the maturation of HSPCs during differentiation.

Conclusion:

Collectively, these findings demonstrated that DEHP exposure selectively reduced SOX2 mRNA expression in human iPSCs, whereas DBP and DINCH did not affect the expression of pluripotency-associated genes. During hematopoietic differentiation, both DEHP and DINCH reduced the number of CD34⁺ cells; however, only DEHP significantly altered subsequent hematopoietic progenitor maturation by increasing CD34⁺CD43⁺CD45⁻ cells and decreasing CD34⁺CD43⁺CD45⁺ cells. These findings suggest that DEHP may disrupt the developmental progression of hematopoietic progenitors toward a CD45⁺ phenotype, whereas DINCH exhibits more limited effects in this human iPSC-based model.

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EFFECTS OF *IN UTERO* EXPOSURE TO AIR POLLUTION ON MATERNAL AND NEONATAL METABOLOMIC PROFILES

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Abstract:

Exposure to traffic-related carcinogenic air pollutants during pregnancy is a significant public health concern due to their potential health risks to both mothers and their newborns. Metabolomics offers a powerful approach to characterize metabolic alterations associated with environmental exposures and to elucidate the biological pathways linking maternal exposure to fetal health. This study aimed to investigate impacts of traffic-related volatile organic compounds (VOCs) on urinary metabolomics profiles, oxidative DNA damage and metabolomics associated biological pathways in mothers and newborns.

A total of 79 mother-newborn pairs participated in the study, residing in high traffic-congested areas (47 pairs) and low traffic density (32 pairs). Urine samples were collected from pregnant subjects throughout the pregnancy period and from the newborns at birth. The levels of targeted urinary VOC metabolites, mandelic acid (MA), phenylglyoxylic acid (PGA), trans, trans-muconic acid (ttMA), and hippuric acid (HA), along with oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OHdG), were measured by HPLC-MS/MS. When compared to low-traffic exposure group, high traffic-exposure mothers had significant higher urinary concentrations of MA at the 1st trimester (120.94 vs 110.90 mg/g creatinine, $p < 0.05$) and ttMA at 2nd trimester (24.74 vs 21.82 mg/g creatinine, $p < 0.05$). Maternal urinary concentrations of PGA and HA were slightly increased in high-exposure group across three trimesters of pregnancy. Maternal urinary 8-OHdG were 1.4-fold significantly higher in the high-exposure group at the 1st trimester ($p < 0.01$). In newborns, high-exposure group had significantly higher concentrations of urinary MA and HA ($p < 0.05$) by 2.26- and 2.03-fold, respectively. Urinary 8-OHdG concentrations were lower in high-exposure newborns.

An untargeted LC-MS/MS-based approach in urinary metabolomics, coupled with spectral library matching, identified a number of VOC-related metabolites with significant differences between exposure groups in both maternal and newborn urine. The metabolomic profiles between maternal and neonatal urine exhibited strong correlations for VOC-related metabolites. Pathway enrichment analysis highlighted considerable metabolic disruptions linked to airborne VOC exposure, mainly impacting amino acid biosynthesis and metabolism in both pregnant women and newborns.

In conclusion, these findings demonstrate that traffic-related VOCs exposure during pregnancy was associated with metabolic perturbations in both mothers and their newborns which may influence fetal metabolic programming. The altered metabolic pathways provide valuable insights for early risk assessment and the development of preventive strategies to improve maternal and newborn health outcomes.

Keywords: Volatile organic compounds, Traffic-related air pollution, oxidative DNA damage, Urinary VOC metabolites, Metabolic pathways

Introduction:

Prenatal exposure to air pollution, particularly traffic-related pollutants such as fine and ultrafine particulate matter (PM_{2.5} and PM_{0.1}), volatile organic compounds (VOCs), PAHs, and toxic metals, has been associated with adverse birth outcomes, including low birth weight and preterm birth, as well as an increased risk of non-communicable diseases. These associations remain incompletely understood (1, 2).

Metabolomics is a powerful approach for characterizing small-molecule metabolites and identifying biochemical alterations induced by environmental exposures. Previous studies have demonstrated that maternal exposure to air pollution during pregnancy disrupts metabolic pathways involved in oxidative stress, inflammation, lipid metabolism, amino acid metabolism, and steroid hormone biosynthesis, which may contribute to impaired fetal growth and development (3-5).

In addition, metabolic alterations related to prenatal air pollution exposure have also been observed in newborns, suggesting that maternal metabolic perturbations may be transmitted to the fetus (6). Therefore, this study aims to investigate the effects of prenatal air pollution exposure on maternal and newborn metabolomic profiles to improve our understanding of the biological pathways linking environmental exposures to early-life health outcomes.

Objectives:

This study aimed to investigate maternal and newborn metabolomic alterations, metabolic pathways, and potential biomarkers associated with prenatal exposure to traffic-related air pollutants.

Methodology:

Study locations

This study was conducted in two areas of Bangkok, Thailand, selected based on traffic density and the availability of local hospitals for participant recruitment. The high-traffic area was located on Somdej Chaophrya Road in Khlongsan District (>2,000 vehicles/day). The low-traffic area was located on Buddhamonthon Sai 3 Road in western Bangkok (< 2,000 vehicles/day).

Sample collection

A total of 79 pregnant women were recruited during the first trimester from participating hospitals, including 47 women residing in high-traffic areas and 32 women from low-traffic areas. Eligible participants were aged 20-40 years, had lived in the study area for at least one year, and had no history of pregnancy complications or major underlying diseases. Maternal urine samples (50 mL) were collected during each trimester of pregnancy, and neonatal urine samples were collected after delivery. All samples were stored at -20°C until analysis.

Part 1 Assessment of VOC exposure and oxidative DNA damage

Determination of targeted VOC metabolites (MA, PGA, ttMA, and HA) in maternal and neonatal urine

Concentrations of targeted urinary VOC metabolites, including HA, MA, PGA and ttMA, were quantified by LC-MS/MS. Urine samples were centrifuged, diluted with Milli-Q water, spiked with d5-PGA as an internal standard, filtered through a 0.22 µm membrane filter, and analyzed using LC-MS/MS. Urinary creatinine was determined by LC-MS/MS for concentration normalization.

Determination of oxidative DNA damage in neonatal urine

Urinary 8-OHdG, an oxidative DNA damage biomarker, was determined using a competitive enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the control of Aging, Japan) according to the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader, and concentrations were quantified based on the standard calibration curve.

Part 2 Investigation of untargeted urinary metabolomics profiles in pregnant subjects and newborns

Determination urinary untargeted VOC metabolites in pregnant subjects and newborns

Untargeted metabolomic profiling of maternal and newborn urine samples was performed using LC-MS/MS. Urine samples were mixed with methanol sulfadimethoxine as an internal standard, vortexed, centrifuged, and the supernatant was transferred to LC-MS/MS vials for analysis. Quality control (QC) samples were prepared by pooling aliquots from all samples and used to assess analytical MS-DIAL for peak detection, alignment, normalization, and metabolite annotation. Metabolites were identified based on accurate mass, retention time, and MS/MS fragmentation patterns using the MS-DIAL spectral library.

Statistical evaluation

Maternal urinary biomarkers were log-transformed and analyzed using linear mixed-effects models (LMMs) to evaluate the effects of exposure group, trimester, and their interaction, accounting for repeated measurements within participants. Significant effects ($p < 0.05$) were further examined using estimated marginal means (emmeans) with Bonferroni adjustment. Neonatal urinary biomarkers were compared between exposure groups using the Mann-Whitney U test. The relationship between maternal and newborn biomarkers will be examined using Spearman's rank correlation to assess the strength and direction of associations. Pathway enrichments were performed using MetaboAnalyst 6.0.

Results and Discussion:

1. Maternal VOC exposure and oxidative DNA damage

Pregnant women in the high-exposure group showed significantly higher levels of the targeted VOC metabolites including MA in the first trimester (190.12 vs 131.66 mg/g creatinine) and ttMA in the second trimester (36.90 vs 25.57 mg/g creatinine), compared with the low-exposure group ($p < 0.05$). Untargeted metabolomics analysis identified significant alterations in several VOC-related metabolites during pregnancy. Catechol and 1-nitropyrene were significantly higher in the high-exposure group than in the low-exposure group in the third trimester, suggesting increased exposure to aromatic hydrocarbons and combustion-related pollutants. In addition, significant trimester-specific changes were observed for catechol, benzene-1,2,4-triol, 4-chlorophenol, 4-bromophenol, and 2-naphthalenol within the same exposure group, indicating dynamic metabolic responses throughout gestation. Urinary 8-OHdG showed significant differences between exposure groups during the first trimester. These findings support a biological link between prenatal exposure to air pollutants, increased oxidative stress, and DNA damage, which may have implications for maternal and fetal health.

2. Neonatal VOC exposure and oxidative DNA damage

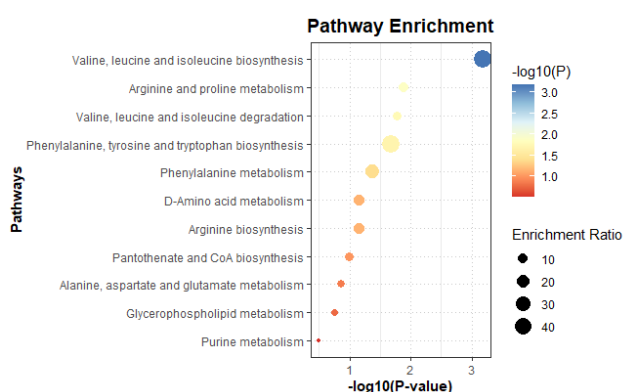
Neonates in the high-exposure group exhibited higher urinary concentrations of the targeted VOC metabolites MA and HA than the low-exposure group, with significant differences observed of MA ($p < 0.1$) and HA ($p < 0.05$). Untargeted metabolomics further revealed significant differences in the VOC-related metabolites for catechol ($p < 0.1$) and benzyl alcohol ($p < 0.05$) between exposure groups. These findings suggest that prenatal exposure to traffic-related air pollutants may influence neonatal VOC-related metabolic profiles and contribute to early-life metabolic alterations. In contrast, urinary 8-OHdG concentrations were significantly higher in neonates from the low-exposure group than in the high-exposure group ($p < 0.1$). This unexpected finding may reflect the limited maturity of neonatal antioxidant, renal and excretory systems, which can affect the production and urinary excretion of oxidative stress biomarkers during the early postnatal period.

3. The associations of metabolomic profiles between mothers and their newborns.

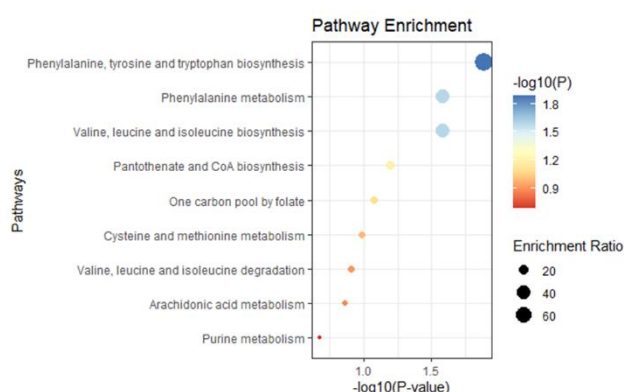
Spearman correlation analysis showed generally weak correlations between maternal urinary metabolites across pregnancy and neonatal urinary metabolites, indicating distinct metabolic regulation between mothers and newborns. Among the metabolites examined, only maternal methionine in the second trimester was significantly positively correlated with neonatal uric acid levels ($p < 0.05$). Given the roles of methionine in one-carbon metabolism and antioxidant defense, and uric acid as an endogenous antioxidant, this association may reflect the influence maternal metabolic status during mid-pregnancy on neonatal redox balance. The overall weak maternal-neonatal correlations suggest that newborn urinary metabolite profiles are shaped not only by maternal metabolism but also by placental regulation, fetal metabolic processes, and postnatal physiological adaptation.

4. Pathway analysis to identify altered biological processes

Pathway enrichment analysis revealed that prenatal VOC exposure was associated with metabolic alterations related to oxidative stress, energy metabolism, and amino acid metabolism in both mothers and newborns. Maternal samples showed enrichment of pathways involved in mitochondrial function and redox homeostasis, whereas neonatal samples exhibited alterations in amino acid, inflammatory, and cellular repair pathways. These findings suggest that prenatal VOC exposure may disrupt metabolic homeostasis and induce adaptive responses in the developing fetus.



Pregnant women



Newborn

Conclusion:

Prenatal exposure to traffic-related air pollutants was associated with altered VOC metabolite profiles, increased oxidative stress, and metabolic pathway perturbations in both mothers and newborns. Maternal-neonatal metabolic correlations further suggest that prenatal exposure may influence the fetal metabolic environment through both exposure-related transfer and metabolic adaptations. These findings provide mechanistic insight into how prenatal air pollution exposure may affect early-life health and development.

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FACTORS AFFECTING HYGIENE BEHAVIOR OF FOOD HANDLERS IN PRIMARY SCHOOLS, MUEANG DISTRICT, PHITSANULOK PROVINCE

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Abstract:

This study was a cross-sectional design study. The objective was to study the factors affecting the hygiene behavior of food handlers in primary schools in Mueang District, Phitsanulok Province. The sample consisted of 90 food handlers in primary schools. The instruments used to collect data from the sample consisted of a questionnaire. The statistics used for data analysis were frequency, percentage, mean, standard deviation, and multiple regression analysis was used to analyze factors influencing food hygiene practices among food handlers. The results of the study revealed that the sample group were females (83.3%), aged 40 - 59 years (51.1%), with more than 10 years of work experience (43.3%), and 54.4% had previously received food hygiene training. The study found that food handlers had a high level of knowledge of food hygiene (93.3%, mean 17.4 ± 1.2), a high level of health beliefs (96.7%, mean 131.7 ± 10.6), and a high level of hygienic behavior (100.0%, mean 88.1 ± 5.7). Multiple regression analysis revealed that chronic diseases, monthly income, and knowledge level significantly predicted hygienic behavior ($R^2 = 0.265$, Adjusted $R^2 = 0.240$, $F = 10.356$, $p < 0.001$), explaining 26.5% of the variance. Health beliefs were not included in the predictive equation. The study highlights the need for continuous training and monitoring. In particular, washing raw materials and separating cutting boards for raw and cooked food are crucial in maintaining and improving hygienic practices and preventing foodborne and waterborne diseases.

Keyword: Food sanitation, Food handlers, Hygiene behavior, Primary schools

Introduction:

Food is a primary necessity for humans. Consuming unclean or contaminated food, such as that containing viruses, bacteria, parasites, chemicals, or heavy metals, can lead to illnesses transmitted through food and water, including acute diarrhea, food poisoning, cholera, dysentery, typhoid fever, and hepatitis A, among others. According to disease surveillance data from the Department of Disease Control, the incidence of acute diarrhea has shown a declining trend over the past 10 years (2015-2024), and has remained stable over the last 5 years, with 1-4 reported deaths per year. In 2025 (January 1 - October 31, 2025), there were 696,225 cumulative reported cases, with an incidence rate of 1,072.56 per 100,000 population and 9 deaths, resulting in a case fatality rate of 0.001% [1]. Meanwhile, the food poisoning situation from 2021 to 2023 showed a continuous upward trend in the incidence rate and an increasing number of outbreaks each year. In 2025 (January 1 - October 31, 2025), there were 122,301 cumulative reported cases, with an incidence rate of 188.91 per 100,000 population, and 1 reported death, with a mortality rate of 0.002 per 100,000 population [2]. From 2021 to 2025, the highest number of outbreaks of acute diarrhea and food poisoning occurred in educational

institutions (190 events), followed by residences (40 events) and communities (28 events). Key risk factors for these diseases include poor sanitation, such as coliform bacteria contamination in utensils and equipment, food handlers' hands, improper food handler hygiene, inappropriate storage of raw materials/food, and unsafe food consumption habits, including consuming unclean drinking water, ice, and tap water [3].

Food sanitation is crucial in managing and controlling factors that cause food contamination, including people, food itself, utensils, equipment, food preparation and sales areas, and disease-carrying animals and insects. It requires cooperation from officials, consumers, businesses, and food handlers in selecting and preparing food to ensure cleanliness and safety for consumers [4]. This is especially important for food handlers involved in all stages of food preparation, cooking, sales, and serving, including the washing and storage of utensils and equipment. Previous studies have shown that knowledge and hygienic behaviors among food handlers are crucial factors in preventing contamination and reducing the risk of foodborne and waterborne diseases. In addition, health belief factors, including perceived seriousness and stimulus-to-act, can predict food hygiene behaviors and promote correct practices among food handlers [5]. And self-efficacy is the most important predictor of food hygiene behavior, followed by perceived benefits, perceived risks, and perceived severity [6].

Health Region 2, comprising Phitsanulok, Tak, Phetchabun, Uttaradit, and Sukhothai provinces, is projected to have the highest number of foodborne and waterborne disease cases in the country by 2025. Outbreaks in educational institutions are continuously observed, particularly in Phitsanulok province, which has reported high rates of diarrhea and food poisoning, with clustered outbreaks occurring in primary schools. Based on the literature review and the aforementioned data, food sanitation among food handlers in schools remains a significant problem, potentially leading to widespread outbreaks of foodborne and waterborne diseases. Most previous studies focused on the knowledge, attitudes, and food sanitation behaviors of food handlers in restaurants, food stalls, and educational institutions. However, other factors, such as health beliefs and perceived self-efficacy, which influence hygienic behaviors among food handlers, lack clear data in the context of primary schools in Phitsanulok Province. Therefore, this research aims to study the factors affecting hygienic behaviors of food handlers in primary schools in Mueang District, Phitsanulok Province. The goal is to provide guidelines for planning, improving, and resolving food sanitation issues to ensure cleanliness, safety, and health benefits for school consumers, as well as to monitor and prevent foodborne and waterborne diseases in schools, enabling rapid and timely responses to such situations.

Methodology:

This research employed a cross-sectional design. The population consisted of 106 food handlers in primary schools in Mueang District, Phitsanulok Province, under the Phitsanulok Primary Educational Service Area Office 1. A total of 90 food handlers who were working in primary schools participated in this research. Simple random sampling was used, selecting at least one food handler from each school to achieve a total sample size of 90 individuals. The research instrument used was a questionnaire consisting of four parts: Part 1: Personal factors, including gender, age, education level, monthly income, chronic diseases, work experience, and food sanitation training. Part 2: Knowledge of food sanitation. Part 3: Health beliefs, including perceived risk of foodborne and waterborne diseases, perceived severity of foodborne and waterborne diseases, perceived benefits of hygienic behavior, perceived barriers to hygienic behavior, and perceived self-efficacy. Part 4: Hygienic behaviors of food handlers. The instrument underwent content validity testing by experts and

was then tested with food handlers in nearby secondary schools to verify its reliability. The results showed that: Food hygiene knowledge, the Kuder-Richardson 20 formula yielded a reliability coefficient of 0.73. Health beliefs, the Cronbach's alpha coefficient yielded a reliability coefficient of 0.89. Hygienic behaviors of food handlers, the Cronbach's alpha coefficient yielded a reliability coefficient of 0.75. The data were analyzed by using SPSS software. The descriptive statistics were used to analyze personal factors, knowledge, health beliefs, and hygienic behaviors of food handlers to determine frequency, percentage, mean, and standard deviation bMultiple regression analysis was used to analyze factors influencing food hygiene practices among food handlers in primary schools in Mueang District, Phitsanulok Province

This research was approved by the Human Research Ethics Committee of the Faculty of Public Health, Mahidol University, as per document COA. NO. MUPH 2026-040 dated March 10, 2026. Data collection was conducted by obtaining permission from the Phitsanulok Primary Educational Service Area Office 1 to collect data from the sample group of schools under their jurisdiction. The objectives of the study were explained, coordination with the schools was carried out, and data collection took place.

Results:

Personal factors

Respondents were mostly female (83.3%), aged 40–59 (51.1%), married (61.1%), with secondary/vocational education (37.8%) and monthly income of 5,000–10,000 baht (45.6%). Most had no underlying disease; 43.3% had over 10 years of experience, and 54.4% had prior sanitation training.

Food sanitation knowledge, Health beliefs, Hygienic behaviors

Food hygiene knowledge: 93.3% of the sample group demonstrated a high level of knowledge in food hygiene (mean 17.4 ± 1.2). The item with the most misconceptions was annual health check-ups (77.8% incorrect answers).

Health beliefs: 96.7% of the sample group demonstrated a high level of health beliefs (mean 131.7 ± 10.6), with high scores in perceived risk, severity, benefits, barriers, and self-efficacy.

Hygienic behaviors: 100% of the sample group exhibited hygienic behaviors (mean 88.1 ± 5.7). The most frequently practiced behavior was washing hands before preparing, cooking, and assembling food (95.6%), while the least frequently practiced behavior was washing fresh meat, vegetables, and fruits before cooking or storing (16.7%).

Factors Predicting Hygienic Behavior

According to the linear regression test, underlying disease, monthly income, and knowledge level jointly predicted hygienic behavior ($R = 0.515$, $R^2 = 0.265$, Adjusted $R^2 = 0.240$, $F = 10.356$, $p < 0.001$), accounting for 26.5% of its variance. Health belief variables were not significant predictors (Table 1).

Table 1 Factors predicting hygiene behavior of food handlers in primary schools.

Predictor	β	p-value	Result
Underlying disease	0.330	0.001	Sig.
Monthly income	0.331	0.001	Sig.
Knowledge level	0.247	0.009	Sig.

Discussion:

The study found that the sample group had high levels of health knowledge, beliefs, and hygienic behaviors. This may be due to having completed food sanitation training courses (54.4%) and having more than 10 years of work experience (43.3%), leading to familiarity with and awareness of the risks of foodborne and waterborne diseases. However, the correlation between high overall scores and specific risky behaviors, such as not separating cutting boards for raw and cooked food and infrequently washing ingredients before cooking, highlights the limitations of assessment using overall scores, which may not clearly reflect specific significant risky behaviors. This aligns with the Health Belief Model, where perceived barriers (e.g., inconvenience in separating cutting boards) may affect adherence to these practices despite high health knowledge and beliefs.

Regarding predictive factors, those with underlying medical conditions and higher incomes tended to have better hygienic behaviors. This may be explained by the fact that those with underlying medical conditions are more aware of the health impacts, while higher income may provide better access to sanitation equipment or resources. While the level of knowledge was statistically significant, its influence was relatively small ($\beta=0.247$), and the three variables together explained only 26.5% of the variance in behavior. This indicates that other factors not included in the model, such as support from school administrators, monitoring measures, or organizational culture, may further influence the behavior of food handlers.

Conclusion:

The sample group had a high level of knowledge regarding food hygiene, health beliefs, and overall hygienic behavior. Chronic diseases, monthly income, and knowledge level were statistically significant predictors of these behaviors. The study results show that, although the overall level was good, there are still specific risky behaviors that need to be addressed, especially the separation of cutting boards for raw and cooked food and the washing of ingredients before cooking. Relevant agencies should therefore conduct workshops that emphasize these specific issues, coupled with continuous monitoring of behaviors, in order to sustainably reduce the risk of foodborne and waterborne diseases in schools.

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IN VITRO MODULATORY EFFECTS OF SODIUM ARSENITE ON CHOLANGIOCARCINOMA-FIBROBLAST INTERACTIONS

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Abstract:

Cholangiocarcinoma (CCA) is an aggressive cancer characterized by a desmoplastic tumor microenvironment (TME), where interactions between cancer cells and stromal cells contribute to cancer progression. Arsenic exposure has been associated with hepatobiliary malignancies, including CCA, but the mechanisms linking arsenic exposure to CCA progression remain poorly understood. This study investigated whether sodium arsenite exposure alters CCA-derived paracrine signaling and the molecular responses of fibroblasts. RNA sequencing was performed on LX-2 cells following direct sodium arsenite exposure or treatment with conditioned media (CM) collected from HuCCA-A4 cells cultured in the presence or absence of sodium arsenite. Our results demonstrated that direct arsenic exposure induced oxidative stress- and inflammation-related pathways in LX-2 cells, while CM from HuCCA-A4 cells promoted a robust inflammatory response in LX-2 cells, with NicheNet analysis prioritizing IL1B-IL1R1 as a potential ligand-receptor interaction. Although NicheNet analysis continue to prioritize IL1B-IL1R1 signaling in LX-2 cells treated with CM from sodium arsenite-exposed HuCCA-A4 cells, these cells exhibited a distinct transcriptional profile characterized by enrichment of protein synthesis, cellular stress adaptation, and proliferative signaling pathways, accompanied by reduced inflammatory signaling. These findings demonstrate that sodium arsenite exposure reshapes CCA-fibroblast communication by modifying cancer cell-derived paracrine signaling and altering fibroblast transcriptional responses. Such changes may contribute to remodeling of the tumor microenvironment and represent a potential mechanism linking environmental arsenic exposure to CCA progression.

Keywords: Cholangiocarcinoma, Arsenic, Sodium arsenite, Cancer-associated fibroblasts, Hepatic stellate cells, Conditioned medium, RNA-seq.

Introduction:

Cholangiocarcinoma (CCA) is an aggressive malignancy of the bile duct epithelium with a poor prognosis (1). In northern and northeastern Thailand, CCA incidence is strongly associated with liver fluke infection (2). However, arsenic exposure, a global public health concern, has emerged as a potential risk factor contributing to hepatobiliary malignancies, including CCA (3, 4). Exposure to arsenic is known to cause oxidative stress, inflammation and fibrosis (an excess accumulation of extracellular matrix (ECM) components within the tissue), however, how arsenic contribute to CCA is unclear.

The tumor microenvironment (TME) plays a critical role in CCA progression. A hallmark feature of CCA is a desmoplastic stroma, a fibrotic environment enriched in ECM components, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and exhausted T cells (5, 6). Interactions between cancer cells and stromal cells are known to contribute to the establishment of the cancer niche, a tumor-supportive microenvironment, thereby facilitating tumor progression and therapeutic resistance (7, 8).

Since arsenic exposure is known to alter the liver microenvironment and is associated with CCA incidence, arsenic exposure may reshape the TME to favor CCA progression. Therefore, this study aims to explore whether arsenic exposure alters CCA-fibroblast communication, particularly its influence on CCA-derived paracrine signaling and the molecular responses of fibroblasts *in vitro*.

Objective:

To investigate the effects of conditioned medium CM containing secreted factors from sodium arsenite-exposed HuCCA-A4 cells on molecular alterations in LX-2 cells.

Methodology:

Cell culture

HuCCA-A4 cells, which are HuCCA-1 cells expressing red fluorescent protein, served as the CCA cell model, while LX-2 cells served as the fibroblast model. Both cells were maintained in culture medium supplemented with fetal bovine serum (FBS), L-GlutaMAX, and penicillin/streptomycin.

Conditioned medium

HuCCA-A4 cells were exposed to 2.5 μ M sodium arsenite for 48 h. CM was subsequently collected, clarified by centrifugation and filtration, and mixed 1:1 with fresh growth medium prior to use. Cell-free control media incubated with or without sodium arsenite under the identical condition were used to represent direct arsenic exposure and its corresponding control. LX-2 cells were subsequently treated with control media, arsenic-containing media, CM from untreated HuCCA-A4 cells, or CM from sodium arsenic-treated HuCCA-A4 cells for 48 h.

RNA extraction, sequencing, and bioinformatic analysis

Bulk RNA-seq was performed in LX-2 cells under four treatment conditions and HuCCA-A4 cells under two treatment conditions as described above. Biological triplicates were prepared for each group. Total RNA was extracted, and RNA concentration and quality were assessed before samples were submitted to a sequencing service provider for library preparation, RNA sequencing and processing using an Illumina platform. RNA-seq count data were analyzed using DESeq2 with the design formula $\sim$ CM + Arsenic + CM: Arsenic. Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA) were performed to identify altered biological processes and signaling pathways. Ligand-receptor interaction was evaluated using NicheNet in R to predict potential HuCCA-A4-derived ligands, LX-2 receptors, and downstream target genes involved in CCA-fibroblast communication.

Results and Discussion:

RNA-seq and pathway enrichment analyses demonstrated that both direct arsenic exposure and CCA-derived paracrine signaling substantially altered fibroblast transcriptional programs. Direct arsenic exposure induced genes and pathways related to oxidative stress and inflammatory signaling, consistent with the known cellular response to arsenic-induced stress. In contrast, HuCCA-A4 CM elicited a pronounced inflammatory phenotype characterized by activation of cytokine-, NF- κ B-, and STAT3-related pathways. As seen in the interaction effect, CM derived from arsenic-exposed HuCCA-A4 cells produced a distinct transcriptional response in LX-2 cells, with enrichment of ribosome biogenesis, protein synthesis, MYC signaling, and unfolded protein response pathways, accompanied by suppression of inflammatory programs. These findings suggest that arsenic exposure alters CCA-fibroblast communication and may promote a molecular and functional shift in fibroblast cells.

Correspondingly, NicheNet-based ligand-receptor analysis predict an alteration in ligand activity from untreated vs sodium arsenite-exposed HuCCA-A4 CM leading to different ligand-receptor communication and transcription changes in LX-2 cells. Notably, IL1B-IL1R1 signaling, a known mediator of inflammatory CAF (iCAF) activation, was prioritized in both conditions. These findings suggest that secreted factors from HuCCA-A4 cells can induce fibroblast activation with features consistent with iCAF phenotypes, while addition of sodium arsenite exposure might not prevent iCAF-like activation but it may lead to distinct fibroblast behavior when compared with a paracrine signaling from untreated HuCCA-A4 cells. Collectively, these findings suggest that sodium arsenite modulates CCA-fibroblast communication by modifying HuCCA-A4-derived paracrine signaling and changing molecular responses in LX-2 cells.

Conclusion:

Sodium arsenite exposure altered fibroblast transcriptional programs through both direct effects and indirect modulation via CCA-derived paracrine signaling. Direct arsenic exposure induced oxidative stress- and inflammation-related responses, whereas secreted factors from HuCCA-A4 cells promoted inflammatory signaling, a characteristic of activated iCAF. Secreted factors from sodium arsenite-exposed HuCCA-A4 cells elicited a distinct transcriptional profile. Taken together, these findings indicate that sodium arsenite can modifies CCA-fibroblast communication and alters the molecular responses of fibroblasts within the CCA microenvironment.

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INTEGRATIVE STUDY OF GENETIC DAMAGE AND TRANSCRIPTOMIC ANALYSIS IN NEWBORNS PRENATALLY EXPOSED TO AIR POLLUTION

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Abstract:

Traffic-related air pollution particularly particulate pollution, fine and ultrafine particulate matters and PAHs, are increasingly recognized for their contribution to potential health risk. Growing evidence suggests that prenatal exposure to air pollution during pregnancy can adversely impact fetal development and increase the likelihood of childhood health problems. This study aimed to investigate genetic damage and transcriptomic alterations in the cord blood of newborns prenatally exposed to traffic-related air pollution. In addition, immunocytochemistry was performed to identify the DNA damage associated with prenatal exposure to air pollution in cord blood and, performed the transcriptomic analysis to identify gene expression alterations and identify molecular pathways potentially involved in DNA damage.

A total of 80 newborns' cord blood samples consisted of 45 high- and 35 low-traffic exposed newborns. Immunocytochemistry detection of DNA damage was performed in cord blood leukocytes to determine 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-nitroguanine (8-nitroG), and benzo[a]pyrene-DNA adduct (BPDE-DNA adduct). When compared to low-exposed group, the mean fluorescent intensity for all DNA damage biomarkers was significantly higher (Adjusted p-value < 0.0001) in the high-exposed group, showing 1.50-fold (88.00 vs 58.56), 1.42-fold (91.46 vs 64.51), and a 1.51-fold (105.92 vs 70.36) for 8-OHdG, 8-nitroG and BPDE-DNA adduct, respectively. RT-PCR analysis of transcription expression of DNA repair gene, *hOGG1*, was 1.62-fold significant reduction (4.07 ± 1.1 vs 6.54 ± 1.4 , $p < 0.0001$) in the high-exposure group. The association between 8-OHdG and *hOGG1* showed a significant negative correlation ($r = -0.3422$, $p < 0.05$). Under the transcriptomic analysis; gene set enrichment analysis (GSEA) revealed that differentially expressed genes were primarily associated with mitophagy, autophagy of mitochondria, myeloid leukocyte activation, leukocyte mediated cytotoxicity, regulation of innate immune response.

In conclusion, the study demonstrates prenatal exposure to traffic-related air pollution was associated with significantly increased DNA damage including 8-OHdG, 8-nitroguanine, and BPDE-DNA adducts, while reducing the *hOGG1* expression in cord blood. Additionally, the transcriptomic findings, together with the increased 8-OHdG levels, suggest that prenatal exposure to traffic-related air pollution induces oxidative DNA damage, and it may contribute to mitochondrial dysfunction, activation of inflammatory and innate immune response pathways. Overall, these findings will help to identify, and clarify mechanistic pathways of developmental toxicity in newborns.

Keywords: Traffic-related air pollution, Prenatal exposure, DNA damage, Gene expression, Transcriptomic

Introduction:

Human health and wellness are basically linked to clean air. However, some human and natural activities contribute to air quality degradation by increasing air pollution. Urbanization, transportation/ traffic emission, motorization, economic development, energy consumption, and an increase in the urban population are such major contributors to causing air pollution (1). Air pollution consists not only of single substances but also of a complex mixture of substances. The effects associated with human exposure may affect by multiple organ systems under different biological mechanisms such as oxidative stress, inflammation, genetic and epigenetic alterations, metabolic activation, and DNA damage and changes in gene expression may influence disease susceptibility later in life (2, 3). Prenatal exposure to air pollution has been associated with effects on embryonic, fetal growth and development, and those effects can arise due to the presence of particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs) (4). Such studies suggest that prenatal exposure to air pollution can induce genetic damage, including DNA strand breaks, oxidative DNA lesions, micronucleus formation, and chromosomal instability in newborns (5). The 8-OHdG, 8-nitroguanine, and BPDE-DNA adducts act as markers of mutagenic genetic damage (6) that can be caused by exposure to air pollution. 8-OHdG indicates oxidative DNA damage, 8-nitroguanine represents nitrosative DNA damage, and BPDE is associated with bulky DNA adduct formation caused by polycyclic aromatic hydrocarbon (PAH) metabolites such as benzo[a]pyrene (6). In addition, the changes in gene expression patterns that can be detected by transcriptomic analysis could show the gene-related functional pathways of DNA damage caused by air pollution. Given these risk, it is necessary to identify gene expression signatures related to oxidative stress, DNA damage response/ DNA repair, inflammation, metabolic activation, detoxification, and cell cycle regulation that are associated with elevated DNA damage levels in newborns prenatally exposed to higher levels of air pollution during pregnancy and it will contribute to better understanding of prenatal air pollution exposure related developmental toxicity and public health concern.

Objectives:

This study aims to investigate genetic damage and transcriptomic alterations in the cord blood of newborns prenatally exposed to traffic-related air pollution.

Methodology:

Study locations

This study was conducted in high- and low-traffic congested areas in Bangkok, Thailand. The initial criteria for selecting the study sites involved assessing traffic density, community demographics and the presence of a hospital to enroll pregnant subjects in the same location. The high-traffic site was located in Somdej Chaophraya Road, Klongsan District, while the low-traffic site was located in peri-Bangkok at Buddhamentol 3 Road.

Sample collection

The current study recruited 80 pregnant women from each hospital at the first trimester of pregnancy. The health status of recruited pregnant subjects was assessed before sample collection. The inclusion and exclusion criteria for subject recruitment were age ranging from 20 to 40 years and residing in the study area for at least 1 year. Umbilical cord blood samples (50 mL) were collected from the umbilical cord immediately after delivery. The cord blood samples (cryopreserved with 10% DMSO) were stored at -80 °C until further analysis.

Part 1 Analysis of DNA damage and DNA repair genes in newborns

Analysis of DNA damage in newborns

DNA damage including 8-OHdG, 8-nitroguanine and BPDE-DNA adducts were analyzed by immunocytochemistry (IHC). Briefly, cryopreserved cord blood samples were thawed, washed, and peripheral blood mononuclear cells (PBMCs) were isolated using the EasySep™ Direct Human PBMC Isolation Kit according to the manufacturer's instructions. The isolated PBMCs were fixed with ethanol, transferred onto chamber slides, and stored at 4 °C until analysis. Prior to IHC staining, the cells were rehydrated and treated with RNase A, chemical denaturation, and proteinase K, followed by sequential incubation with primary antibodies against 8-OHdG, 8-nitroguanine, and BPDE-DNA adducts, and the corresponding fluorescent secondary antibodies. After DAPI counterstaining and mounting, fluorescence images were acquired using the TissueFAXS PLUS 6.0 imaging system, and immunofluorescence was quantified with TissueQUEST 6.0 software by analyzing 1,000 cells per sample.

Transcript expression of DNA repair genes

To isolate total RNA from umbilical cord blood, the RNeasy Mini Kit was used following the manufacturer's instructions. After isolation of total RNA, the concentration of RNA in each sample was determined using a NanoDrop spectrophotometer. Then, 2000 ng/μl of RNA was reverse-transcribed into cDNA to perform PCR-array and RT-qPCR. This synthesized cDNA was subsequently used for PCR array analysis of DNA repair genes and RT-qPCR analysis to further validate the DNA repair ability for selected DNA repair genes, including *hOGG1* and *XRCC1*, using SYBR Green chemistry.

Part 2 Transcriptomic analysis

For RNA sequencing, determine the RIN number of isolated RNA before sending the RNA to the company. Agilent RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer was used to determine RNA integrity number (RIN) following the manufacturer's Quick Start Guide instructions. The RNA samples were then submitted to Novogene Co., Ltd. for RNA sequencing and downstream bioinformatics analysis. After receiving the RNA sequencing data, R software (version 4.4.2) was used to conduct the transcriptomic analysis under bioinformatics analysis.

Statistical evaluation

Statistical analyses were performed using R software (version 4.4.2). Data were expressed as mean ± standard deviation (SD). Transcriptomic data were processed using a standard RNA-seq analysis pipeline, including quality control of raw reads, trimming, alignment to the reference genome, gene-level quantification, normalization, and differential expression analysis using R software. Mann-Whitney U test was used to evaluate statistical differences between groups. A p-value < 0.05 was considered the differences with a statistically significant. Spearman's correlation coefficient (r) was used to evaluate the association of the study parameters. Statistical analysis of the data was performed using GraphPad Prism 9.3.0 (463).

Results and Discussion:

1.1 Image analysis of DNA damages in newborns' cord blood

Images from immunocytochemistry identified 8-OHdG was visualised in green fluorescence, 8-nitroguanine in yellow fluorescence, and BPDE-DNA adducts in pink fluorescence. The stronger fluorescence intensity observed in the high-exposed group compared with the low-exposed group, based on visual observation via microscope, suggests

increased accumulation of DNA lesions in nuclear DNA. Therefore, the merged images provide visual evidence that oxidative, nitrative, and PAH-induced DNA damage occurred predominantly within the cell nuclei.

1.2 Analysis of DNA damage in newborns' cord blood

The mean fluorescent intensity for all DNA damage biomarkers was significantly higher in the high-exposed group, showing 1.50-fold (88.00 vs 58.56, $p < 0.001$), 1.42-fold (91.46 vs 64.51, $p < 0.001$), and a 1.51-fold increase (105.92 vs 70.36, $p < 0.001$) of fluorescent intensity for 8-OHdG, 8-nitroG and BPDE-DNA adduct, respectively.

1.3 Transcript expression of DNA repair genes

There was a 0.62-fold significant reduction in *hOGG1* gene expression in the high-exposed group compared with the low-exposed group (4.07 ± 1.1 vs 6.54 ± 1.4 , $p < 0.0001$), and this finding suggests that reduced *hOGG1* expression may impair the removal of oxidative DNA lesions such as 8-OHdG. Consequently, it may contribute to the accumulation of DNA damage observed in exposed individuals. The association between 8-OHdG and *hOGG1* showed a negative correlation ($r = -0.3422$) with a significant relationship between each other (p -value = 0.0019).

2 Transcriptomic analysis

Results obtained from GO enrichment analysis showed that upregulated genes were primarily associated with myeloid cell homeostasis, erythrocyte homeostasis, erythrocyte differentiation, cellular transport, haemoglobin metabolism and biosynthesis and cytoskeletal organization, while extracellular matrix organization, taxis, chemotaxis, and leukocyte chemotaxis pathways were enriched with downregulated genes. GSEA further identified significant enrichment of pathways involved in mitophagy, autophagy in mitochondrial, and immune regulation in the high-exposure group via myeloid leukocyte activation and leukocyte-mediated cytotoxicity. These findings suggest that prenatal exposure to air pollution induces widespread transcriptional reprogramming, disrupting pathways involved in cellular homeostasis, extracellular matrix integrity, immune regulation, and stress responses, which may contribute to increased DNA damage and impaired DNA repair in newborn cord blood cells.

Conclusion:

This study reported that prenatal exposure to traffic-related air pollution was associated with significantly increased oxidative, nitrative, and PAH-induced DNA damage in newborn cord blood cells, as demonstrated by elevated levels of 8-OHdG, 8-nitroguanine, and BPDE-DNA adducts. This increased DNA damage was accompanied by reduced expression of DNA repair genes, particularly *hOGG1*. These results suggest impaired base excision repair capacity and accumulation of oxidative DNA lesions. Transcriptomic analysis further revealed widespread gene expression changes, while GO enrichment and GSEA identified altered biological pathways related to erythrocyte function, protein synthesis, mitochondrial quality control, immune regulation, extracellular matrix organization, and cell adhesion. Overall, this study provides molecular evidence that prenatal air pollution exposure may contribute to adverse health outcomes in newborns by inducing persistent genomic and transcriptomic alterations. Those approaches may help identify early biomarkers of exposure, clarify mechanistic pathways of developmental toxicity, and ultimately, those findings may contribute to improved public health and to the development of risk assessment and preventive strategies.

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MULTIDRUG RESISTANCE REGULATION BY TetR-type REGULATOR AmcR IN *S. maltophilia*

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Abstract:

Antimicrobial resistance (AMR) represents a critical global threat and remains one of the major challenges in public health driven by the rising number of ineffective antimicrobial agents. This study focuses on *Stenotrophomonas maltophilia*, an opportunistic pathogen that can cause nosocomial infections. It is known as a multidrug-resistant bacterium due to its intrinsic resistance to multiple broad-spectrum antibiotics and its ability to acquire novel antibiotic resistance traits. The *amcR* has been identified in *S. maltophilia*, located upstream of *amcP1* gene, encoding a putative adaptor protein and *amcP2* encoding a putative multidrug efflux pump protein belonging to the resistance nodulation and cell division (RND) superfamily, suggesting an operonic structure. The *amcR* encodes a TetR-type transcriptional repressor, which is associated with the regulation of genes involved in bacterial antimicrobial resistance through one-component signal transduction system. A molecular docking study was performed with the most active compounds, including several antibiotics against *S. maltophilia* AmcR protein. The potential binding affinity of the ligands to the AmcR protein are ciprofloxacin, ceftazidime, amoxicillin and trimethoprim with CNN affinity score (pK) of 6.879, 7.532, 6.604 and 6.616, respectively. Deletion of *amcR* resulted in increased resistance of *S. maltophilia* to several antibiotics, such as, levofloxacin, ciprofloxacin, tetracycline, minocycline, ceftazidime and amoxicillin-clavulanic acid. These findings suggest that the absence of AmcR increased antibiotic resistance in *S. maltophilia*, which possibly due to upregulation of AmcP1-AmcP2 efflux pumps.

Keywords: *Stenotrophomonas maltophilia*, AmcR, Antimicrobial resistance (AMR), TetR-type regulator

Introduction:

Stenotrophomonas maltophilia is an opportunistic pathogen responsible for nosocomial infections in immunocompromised patients. The difficulty of treatment infections caused by *S. maltophilia* is due to the intrinsic resistance to multiple broad-spectrum antibiotics and its capacity to acquired antibiotic resistant traits through horizontal gene transfer. *S. maltophilia* is widely considered as a multidrug-resistant pathogen (MDR), frequently mediated by over-production of efflux pumps of which, majority belonging to RND type. These pumps tend to have a diverse range of substrates, such as quinolones, tetracycline, chloramphenicol, erythromycin, aminoglycosides, and β -lactams (1). Genome analysis of *S. maltophilia* K279a has revealed that *amcP1* and *amcP2* encoding the RND type efflux pump, which plays a role in the intrinsic resistance of *S. maltophilia* to multiple class of antibiotics. *amcR* gene encodes AmcR protein, a putative TetR-type transcriptional regulator, and is located immediately upstream of the co-directionally transcribed *amcP1* and *amcP2* genes. Therefore, the AmcR has been proposed to regulate the expression of AmcP1 and AmcP2 pumps. The regulatory role of AmcR on *amcP1* and *amcP2* expression was elucidated in this study.

Methodology:

Docking studies

The starting three-dimension (3D) structure of *S. maltophilia* AmcR was obtained from the ESMFold tool. Ligand molecules were obtained in SMILES format from PubChem database. Molecular docking was carried out using molecular docking pipeline (available at https://github.com/SunshineDalgarno1/autodock_shasha), which incorporate P2Rank, a prediction and evaluation of multiple binding pockets software and GNINA a molecular docking tool (2, 3). Visualization of models were done by PyMOL software.

amcR deletion mutant construction

The *amcR* deletion mutant was constructed using NEBuilder Hifi DNA Assembly Cloning Kit. The DNA fragments of the *amcR* amplified from *S. maltophilia* chromosome and the fragments amplified from pCM351 vector containing *loxP* region and gentamicin (GM) cassette were assemble onto pKNOCK-Chloramphenicol (CM). The recombinant plasmid then transferred into *Escherichia coli* BW20767, creating pCM Δ *amcR*: : Gmlox. Creation of AmcR deletion mutant of *S. maltophilia* was performed by transformation of plasmid pCM Δ *amcR*: : Gmlox into *S. maltophilia* by conjugation. Double-crossover homologous recombination generated mutants with gene replacement that were selected by screening for gentamicin-resistant phenotypes and confirmed by PCR, resulting in Δ *amcR*: : Gmlox strains. Excision of the GM cassette was achieved using the *Cre-lox* system. The Δ *amcR*: : Gmlox mutant was transformed with pCM157 plasmid containing a Cre-recombinase gene, which mediated cleavage of the *loxP* region out. Gentamicin-sensitive transformants were selected and confirmed by PCR (4).

Disk diffusion assay

Disk diffusion assay was performed using Kirby-Bauer disk diffusion susceptibility test protocol (5). Briefly, overnight cell cultures were diluted with 0.9% saline solution and spread on Mueller-Hinton Agar (MHA) plate. Antibiotic disks were laid on the solidified agar plate and incubated for at least 20 hours at 35°C before inhibition zone measurement.

Result and Discussion:

Molecular docking of AmcR protein with antibiotics

Molecular docking results against *S. maltophilia* AmcR protein indicated an affinity for ligand binding (Table1). The scoring was evaluated by 4 main scoring categories based on the GNINA scoring system; the physics-based binding energy Vina affinity (<-6 kcal/mol), intramolecular energy strain (<2 kcal/mol), a deep learning model based for ligand pose prediction CNN pose score (>0.3) and prediction of binding affinity CNN affinity (>5 pK) using a more flexible approach to simulate real biological interactions (6). Based on the molecular docking result, the potential ligands for AmcR binding are ciprofloxacin, ceftazidime, amoxicillin and trimethoprim with CNN affinity score of 6.879, 7.532, 6.604 and 6.616, respectively. We hypothesized that AmcR is a regulator of efflux pump in *S. maltophilia*. Construction of *amcR* deletion allele was introduced into *S. maltophilia* K279a, generating the Δ *amcR* mutant to investigate the regulatory effect of AmcR.

A

Drug name	Vina Affinity (kcal/mol)	Intramol Energy (kcal/mol)	CNN Pose Score	CNN Affinity (pK)
Ciprofloxacin	-6.25	0.37	0.3465	6.879
Ceftazidime	-6.02	0.28	0.4392	7.532
Amoxicillin	-7.72	-0.5	0.3338	6.604
Trimethoprim	-6.42	-0.38	0.503	6.616

B

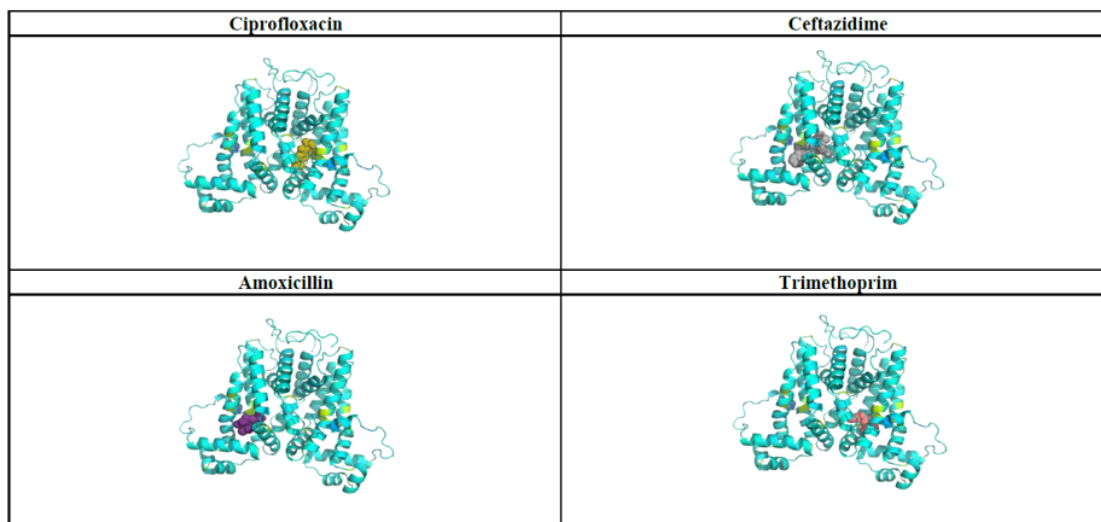


Table 1 Molecular docking for ligand binding prediction of AmcR. (A) GNINA scoring for compounds based on the molecular pipeline filter. (B) Visualization of AmcR with ligands from molecular docking results.

The antibiotic susceptibility of $\Delta amcR$ mutant

Disk diffusion assay showed that $\Delta amcR$ mutant exhibited an increased resistance to levofloxacin, moxifloxacin, ciprofloxacin, tetracycline, minocycline, tigecycline, ceftazidime and amoxicillin-clavulanic acid, comparing to wild-type strain (**Figure 1**).

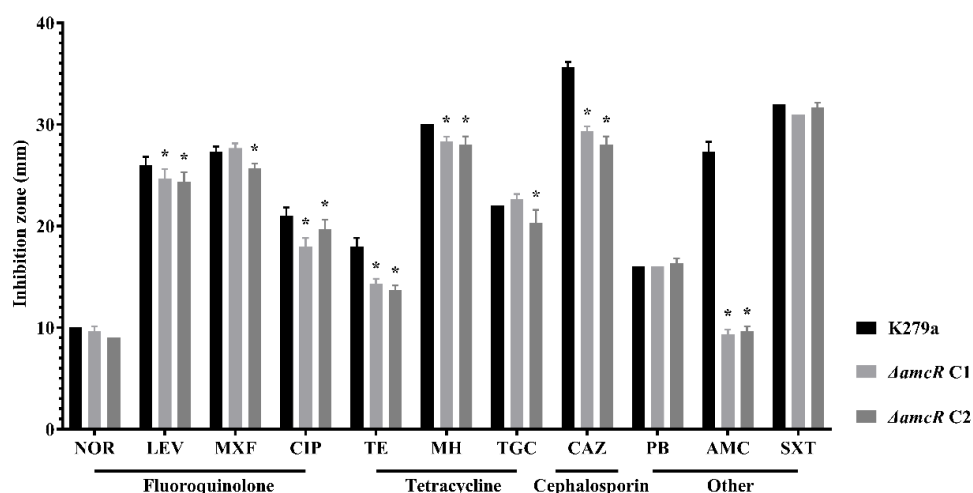


Figure 1 Antibiotic disk diffusion assay of $\Delta amcR$ mutant; fluoroquinolones; Norfloxacin (NOR), Levofloxacin (LEV), Moxifloxacin (MXF), Ciprofloxacin (CIP), tetracycline; Tetracycline (TE), Minocycline (MH), Tigecycline (TGC), cephalosporin; Ceftazidime (CAZ), other antibiotics; Polymyxin B (PB), Amoxicillin-clavulanic acid (AMC) and Trimethoprim/ sulfamethoxazole (SXT). Results shown are mean $\pm$ SDs from three independent experiments. The * represented statistically significant difference using 2-way ANOVA test with $P < 0.05$.

Based on the phenotypic test and the typical regulatory function of transcription repressor TetR that represses its target gene, efflux pump *tetA*, the significant increased resistant phenotype to multiple antibiotics had been observed in $\Delta amcR$ mutant, suggesting that AmcR functions as regulatory repressor like TetR regulator similar to SmeT that act as a repressor for SmeDEF multidrug efflux pump (7). Based on the gene organization, *amcR* and *amcP1* and *amcP2* are co-transcribed as an operon (Figure 2), thus AmcR is likely involved in regulating *amcP1* and *amcP2*. In the presence of antibiotic, it would bind to AmcR, resulting in derepression of AmcR from its promoter region and subsequent the transcription of *amcP1* and *amcP2*.



Figure2 Gene organization of *amcR* associated genes, *amcR*, *amcP1* and *amcP2* in *S. maltophilia*

Conclusion:

Deletion of *amcR* resulted in increased antibiotics resistance in *S. maltophilia*. This finding suggests that AmcR plays an important role in antibiotic resistance as a transcriptional repressor. Based on the gene organization, *amcR* located directly upstream of *amcP1* and *amcP2*, it likely functions as the key regulator of this *amcP1* and *amcP2* efflux pump. Molecular docking analysis suggested that antibiotics such as ciprofloxacin, ceftazidime, amoxicillin and trimethoprim may act as potential effector of AmcR. However, further investigation is required to characterize the regulatory roles of AmcR and its contribution to efflux-mediated resistance in *S. maltophilia*.

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ORGANIZATION CARBON FOOTPRINT ASSESSMENT OF PROMKIRI HOSPITAL, NAKHON SI THAMMARAT PROVINCE

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Abstract:

This research aimed to assess the organizational carbon footprint of Promkiri Hospital, Nakhon Si Thammarat Province. Activity data related to greenhouse gas (GHG) emissions were collected to estimate carbon footprint for fiscal year 2025. GHG emission factors from the Thai Greenhouse Gas Organization (TGO) and relevant publications were applied. The emission sources were categorized into three scopes: direct GHG emissions (Scope 1), indirect GHG emissions from energy consumption (Scope 2), and other indirect GHG emissions (Scope 3). The results showed that the total carbon footprint amounted to 464 tCO₂eq. Among the three scopes, Scope 2 accounted for the largest share of emissions at 232 tCO₂eq, representing 50.22% of the total emissions and attributable mainly to the hospital's electricity consumption. This was followed by Scope 3, which generated 174 tCO₂eq, representing 37.66% of the total emissions and driven primarily by employee commuting. Scope 1 contributed 56 tCO₂eq, representing 12.12% of the total emissions and was mainly associated with fugitive emissions. The findings of this study will serve as a baseline for developing strategies to reduce GHG emissions from the hospital's operations and support the transition toward a low-carbon hospital.

Keywords: Climate change, Global warming, Hospital, Carbon footprint, GHG emissions

Introduction:

Climate change, driven by greenhouse gas (GHG) emissions from human activities since the nineteenth century, has generated extensive direct and indirect consequences for public health. Direct impacts include increased morbidity and mortality associated with heat waves, heat stroke, cardiovascular diseases, and injuries and fatalities resulting from extreme weather events such as floods, storms, and droughts. These impacts are further exacerbated by worsening air pollution as global temperatures continue to rise. Indirect impacts include the geographic and seasonal expansion of vector-borne diseases, such as malaria and dengue fever, the increased incidence of waterborne and foodborne diseases, including cholera, and rising rates of malnutrition resulting from declining agricultural productivity (WHO, 2003). Global GHG emissions currently amount to approximately 53.2 GtCO₂eq (European Commission, 2024). Although the healthcare sector is fundamentally responsible for protecting and promoting public health, it paradoxically contributes approximately 4.4% of global greenhouse gas emissions (Health Care Without Harm & Arup, 2019), equivalent to roughly 2.0 GtCO₂eq. High-income countries account for the largest shares of healthcare-related emissions, with the United States (7.6%), Switzerland (6.7%), and Japan (6.4%) reporting the highest proportions of national GHG emissions attributable to the healthcare sector. Furthermore, approximately 71% of healthcare-related emissions originate not from direct clinical operations but from upstream supply chain activities, including pharmaceutical manufacturing and transportation, medical equipment production, food procurement, and energy generation (Feteira-Santos, 2024). This pattern is well illustrated by Japan, where input–output analyses revealed that healthcare-related emissions increased from 62.5 to 72.0

million tCO₂eq between 2011 and 2015, representing an increase from 4.6% to 5.2% of the country's total GHG emissions. These emissions were driven primarily by medical services and pharmaceutical dispensing, while inpatient care generated more than five times the emissions per patient compared with outpatient care (Nansai et al., 2020). These findings demonstrate the substantial potential for GHG emission reductions through improvements in healthcare service delivery and supply chain management.

Despite growing interest in healthcare decarbonization, existing studies have focused predominantly on secondary- and tertiary-level healthcare facilities. In contrast, community hospitals, which serve as the primary point of contact between the healthcare system and the public by providing health promotion, disease prevention, primary medical care, and rehabilitation services, remain largely unexplored with respect to their organizational carbon footprint (SERI, 2023). Consequently, a systematic assessment of GHG emissions from community hospitals is of considerable academic and policy importance, as it provides an essential evidence base for developing effective and practical emission reduction strategies within Thailand's public healthcare sector (United Nations Thailand, 2025). Therefore, this study aimed to assess the organizational carbon footprint of Promkiri Hospital, Nakhon Si Thammarat Province, Thailand. The findings will provide baseline GHG emission data to support the development of policies and strategies for reducing emissions and advancing low-carbon healthcare operations.

Materials and method:

This study employed an analytical survey research design. Resource and energy consumption associated with various hospital activities that generate GHG emissions was examined. The emission sources were categorized into three scopes: direct GHG emissions (Scope 1), indirect GHG emissions from energy consumption (Scope 2), and other indirect GHG emissions (Scope 3). Activity data collected from these sources were used to calculate the hospital's organizational carbon footprint. GHG emission factors published by the TGO and other relevant sources were applied in the assessment. The carbon footprint was quantified in accordance with the Corporate Carbon Footprint (CFO) assessment guidelines established by the TGO, and the results were expressed in ton of carbon dioxide equivalent (tCO₂eq) per year (TGO, 2018). The emission sources of greenhouse gas (GHG) at Promkiri Hospital are presented in Table 1

Table 1 Emission sources of greenhouse gas from Promkiri Hospital

Emission sources	Details
Scope 1: Direct missions	1.1 Stationary combustion - Diesel fuel for lawn mowers and backup power generators - Liquefied petroleum gas (LPG) for kitchen and food preparation activities 1.2 Mobile combustion - Diesel fuel for ambulances and other hospital vehicles 1.3 Fugitive emissions - Carbon dioxide (CO₂) from fire suppression systems - Methane (CH₄) emissions from septic tank systems - Refrigerant leakage from air-conditioning systems using R-32
Scope 2: Energy Indirect Emissions	Electricity consumption

Emission sources	Details
Scope 3: Other Indirect Emissions	3.1 Purchased goods and services - Municipal water consumption - A4 paper consumption - Garbage bag - Pharmaceutical products, including Amlodipine (AMBES-5), Gabapentin GPO (Gabapentin 100 mg), and Omeprazole GPO 20 mg - Medical supplies, including latex examination gloves and medical face masks 3.3 Fuel and Energy related activities - Upstream emissions associated with purchased electricity - Upstream emissions associated with LPG procurement - Upstream emissions associated with diesel fuel procurement 3.5 Waste generated in operations - General waste - Infectious waste - Hazardous waste 3.6 Business travel - Air travel - Car travel 3.7 Employee commuting - B7 diesel fuel for commuting by private car and public bus - E10 gasohol for commuting by private car and motorcycle - E20 gasohol for commuting by private car - Gasoline (91/95) for commuting by private car - Electric vehicles for commuting

Results and Discussion:

The total GHG emissions from Promkiri Hospital were estimated at 464 tCO₂eq per year. As shown in Figure 1, Scope 2 (electricity consumption) was the largest source of emissions, accounting for 232 tCO₂eq per year, or 51% of the total GHG emissions. Scope 3 which included purchased goods and services, fuel and energy related activities, waste generated from operations, business travel, and employee commuting, was the second-largest source, contributing 174 tCO₂eq per year (37.50% of total GHG emissions). Scope 1, including stationary combustion, mobile combustion, and fugitive emissions, accounted for 56 tCO₂eq per year, representing 12.07% of the total GHG emissions. In addition, biogenic CO₂ emissions, which are reported separately from the three GHG emission scopes, amounted to 2.0 tCO₂eq per year, representing 0.43% of the total emissions.

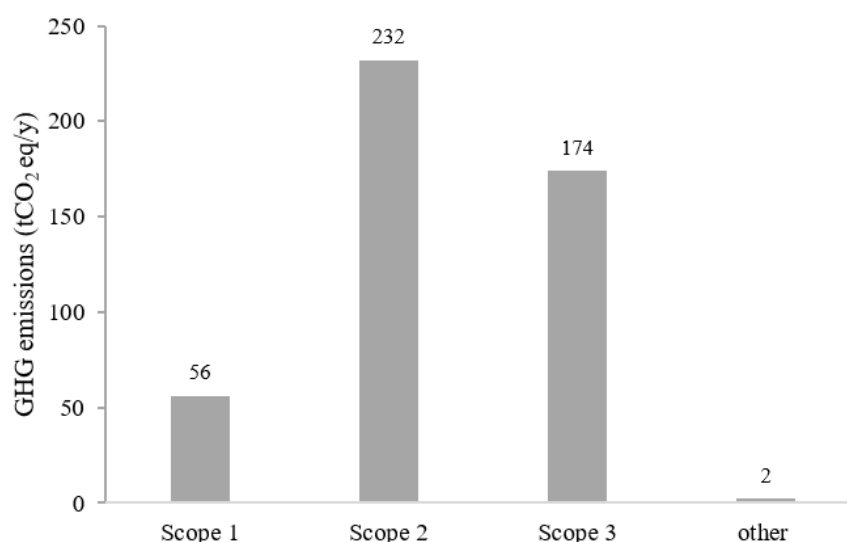


Figure 1 GHG emissions from Promkiri Hospital

Total Scope 1 GHG emissions amounted to 56 tCO₂eq per year. Fugitive emissions from refrigerants were the largest contributor, accounting for 26.19 tCO₂eq per year (46.8% of total Scope 1 emissions). Fuel combustion in mobile sources contributed 24.64 tCO₂eq per year (44.0%), while fuel combustion in stationary sources accounted for 4.41 tCO₂eq per year (7.9%). Scope 2 emissions, derived entirely from purchased electricity consumption, totaled 232 tCO₂eq per year and represented the largest source of GHG emissions, accounting for 51% of the hospital's total emissions (Figure 2). This finding reflects the energy-intensive nature of hospital operations, which require the continuous operation of medical equipment, air conditioning systems, lighting, and other electrical devices to support patient care and essential healthcare services. Scope 3 GHG emissions at Promkiri Hospital were assessed across five categories, with total emissions amounting to 173.52 tCO₂eq per year. Category 3.7 (Employee Commuting) was the largest contributor, accounting for 57.88 tCO₂eq/year. The dominant source within this category was E10 gasohol consumed by employees commuting by private car, followed by gasoline (12.06 tCO₂eq/year) and B7 diesel used for private car commuting (8.62 tCO₂eq/year). Category 3.1 (Purchased Goods and Services) was the second-largest contributor, generating 45.85 tCO₂eq/year, with garbage bags, latex examination gloves, and water consumption identified as the major emission sources. Category 3.3 (Fuel- and Energy-Related Activities) contributed 43.64 tCO₂eq/year, primarily from upstream emissions associated with purchased electricity, followed by upstream diesel and liquefied petroleum gas (LPG) procurement. Category 3.5 (Waste Generated in Operations) accounted for 17.87 tCO₂eq/year, with general waste contributing the largest share, followed by infectious waste and hazardous waste. Category 3.6 (Business Travel) generated 8.28 tCO₂eq/year, comprising emissions from air and road travel.

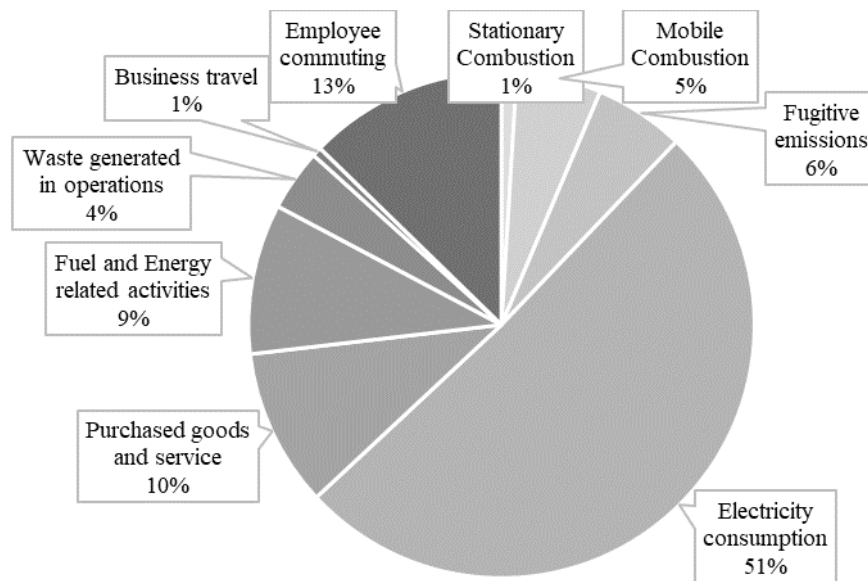


Figure 2 Distribution of GHG emissions by emission sources

However, a comparison of GHG emissions reported by Promkiri Hospital and other hospitals is presented in Table 2. The findings indicate that the carbon footprint varies according to hospital size, operational characteristics, and the scope of GHG accounting. Among the four hospitals, Ratchaphiphat Hospital, the largest facility with 177 beds, reported the highest total GHG emissions at 2,027.82 tCO₂eq/year (Sriapai & Phoochinda, 2021). However, the Scope 3 assessment in that study was limited to Category 3.1 (purchased goods and services, including water and paper consumption) and Category 3.5 (waste generated in operations), which may have resulted in an underestimation of indirect emissions compared with studies adopting a more comprehensive Scope 3 assessment. Buayai Hospital reported total GHG emissions of 1,852 tCO₂eq/year (Kongritti et al., 2025), whereas Krongpinang Hospital reported 560 tCO₂eq/year (Jijai et al., 2023). The dominant emission source also differed among hospitals. Purchased electricity (Scope 2) was the largest contributor at both Ratchaphiphat and Buayai hospitals, accounting for 48.43% and 77.0% of total GHG emissions, respectively. In contrast, Scope 1 emissions were the principal source at Krongpinang Hospital, contributing 51.26% of total GHG emissions. Total Scope 1 emissions at Krongpinang Hospital amounted to 287.17 tCO₂eq/year in 2022, of which fugitive emissions from R-22 refrigerant leakage from air-conditioning systems accounted for 246.30 tCO₂eq/year (85.77%). Diesel consumption in hospital vehicles was the second-largest source (35.05 tCO₂eq/year; 12.20%), followed by liquefied petroleum gas (LPG) used in the hospital kitchen (3.39 tCO₂eq/year; 1.18%), diesel consumption for backup generators (2.17 tCO₂eq/year; 0.75%), and gasohol consumption by lawn mowers (0.26 tCO₂eq/year; 0.09%). Compared with the other hospitals, Promkiri Hospital exhibited a relatively high proportion of Scope 3 emissions, accounting for 37.5% of total GHG emissions. This reflects the inclusion of a broader range of Scope 3 categories in accordance with the GHG Protocol, including purchased goods and services, fuel- and energy-related activities, waste generated in operations, business travel, and employee commuting. These findings demonstrate that differences in organizational boundary setting and the selection of Scope 3 categories can substantially influence both total GHG emissions and the relative contribution of individual emission sources. Therefore, harmonized GHG accounting boundaries and consistent reporting methodologies are essential for meaningful comparisons of carbon footprints across healthcare facilities.

Table 2 Comparison of organizational carbon footprint among Thai hospitals

Study	Baseline Year (Fiscal Year)	GHG Emissions (tCO ₂ eq/year)	Number of Beds	Intensity (tCO ₂ eq/bed)
1. Promkiri Hospital, Nakhon Si Thammarat Province (This study)	2568	464.00	30	15.47
2. Buayai Hospital, Nakhon Ratchasima Province	2567	1,852.0	170	10.89
3. Krongpinang Hospital, Yala Province	2566	560.25	51	10.99
4. Ratchaphiphat Hospital, Bangkok	2562	2,027.8	177	11.46

Conclusion:

Total GHG emissions from Promkiri Hospital, Nakhon Si Thammarat Province, during fiscal year 2025 were 464 tCO₂eq. Among the three emission scopes, Scope 2 was the largest contributor, accounting for 232 tCO₂eq (50.00%) of total emissions, primarily due to electricity consumption. Scope 3 was the second-largest contributor, generating 174 tCO₂eq (37.50%), with employee commuting identified as the major emission source. Scope 1 accounted for 56 tCO₂eq (12.07%), with fugitive emissions from refrigerant leakage representing the largest share within this scope. These findings provide an important baseline for GHG emissions in Thai community hospitals and offer a valuable reference for future benchmarking. Furthermore, the results can support the development of targeted, health facility-level GHG mitigation strategies and contribute to efforts to reduce carbon emissions within Thailand's public healthcare sector.

Acknowledgements:

The authors would like to express their sincere gratitude to the Center of Excellence on Environmental Health and Toxicology (EHT), Office of the Permanent Secretary (OPS), Ministry of Higher Education, Science, Research and Innovation, for its excellent technical support. The authors also extend their sincere appreciation to the staff of Promkiri Hospital for providing the necessary data and for their valuable cooperation throughout the study, which made this research possible.

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REFINEMENT AND SENSITIVITY ASSESSMENT OF A LAMP ASSAY FOR DETECTING wAlbB *WOLBACHIA* IN *Aedes aegypti*

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Abstract:

Mosquito-borne diseases transmitted by *Aedes aegypti*, including dengue, chikungunya, Zika, and yellow fever, remain major public health concerns. As rising global temperatures may expand mosquito vector ranges, effective vector control and monitoring strategies are increasingly important. This study developed and optimized a loop-mediated isothermal amplification (LAMP) assay for detecting the wAlbB *wsp* gene in wAlbB-transinfected Thai *Ae. aegypti*. The optimized assay used 66°C and a 30-min incubation time, detecting *wsp* standard DNA at 100 pg/μL. Matrix spike assays confirmed successful *wsp* detection in mosquito rearing water, larvae, and adult female extracts, with no amplification in non-spiked controls. The assay also detected wAlbB *wsp* directly from crude DNA extracted from a single transinfected mosquito within 20 min. These findings suggest that LAMP is a simple, rapid preliminary method for *Wolbachia* detection and could be further developed for field monitoring applications.

Keywords: *Aedes aegypti*; *Wolbachia*; wAlbB; Loop-mediated isothermal amplification

Introduction:

Aedes aegypti is a major vector of arboviruses, including yellow fever, dengue, chikungunya, and Zika viruses [1]. Rising global temperatures may expand its geographic range and increase mosquito-borne disease risk [2], making *Ae. aegypti* an important target for vector control. *Wolbachia*-based technology offers a promising and environmentally friendly approach by suppressing arbovirus replication and inducing cytoplasmic incompatibility, in which mating between *Wolbachia*-infected males and uninfected females results in embryonic mortality [3,4]. Following the release of wAlbB-transinfected mosquitoes, monitoring *Wolbachia* infection is essential for assessing its prevalence, persistence, and spread in mosquito populations. Although PCR-based methods are widely used, they require specialized laboratory equipment. In contrast, loop-mediated isothermal amplification (LAMP) provides a simpler alternative by amplifying DNA under isothermal conditions using only a constant-temperature heat block or water bath [5,6]. Therefore, this study aimed to refine and optimize a LAMP-based assay for detecting wAlbB infection as a rapid screening tool to support *Wolbachia*-based mosquito control programs.

Methodology:

Preparation of DNA samples

For preparation of the positive control standard, the *wsp* gene was amplified from genomic DNA extracted from wAlbB-transinfected Thai *Ae. aegypti* using PCR with the wAlbB *wsp* primers 81F and 691R. [7]. The amplicon was sequence-confirmed, cloned into the pGEM®-T Easy Vector System, transformed into *E. coli* DH5 α , and purified using the PureDireX Plasmid miniPREP Kit (Bio-Helix, Taiwan). The resulting recombinant plasmid was diluted to a concentration of 10 ng/ μ L and stored at -20°C . This plasmid was used as the *wsp* standard DNA in subsequent experiments.

LAMP primers selection and verification

The LAMP primer set targeting the wAlbB *wsp* gene was obtained from Thayanukul and Lertanantawong et al. [8]. Primer specificity was verified by aligning the target region with the *wsp* sequence of wAlbB-transinfected Thai *Ae. aegypti* available in GenBank (NCBI accession no. MZ325222) and with the *wsp* amplicon sequence generated in this study. In addition, the primer sequences were analyzed using BLAST to assess potential off-target matches. The analysis confirmed that the primers specifically target the wAlbB *wsp* region and were suitable for subsequent LAMP assays.

LAMP optimization and sensitivity assay

The LAMP assay was optimized for detection of the wAlbB *wsp* gene in a total reaction volume of 10 μ L. The reaction mixture was adapted from Thayanukul and Lertanantawong et al. [8] and contained 1 $\times$ isothermal buffer, 2 mM MgSO $_4$, 1.4 mM dNTPs, 0.8 M betaine, 1.6 μ M each of the inner primers FIP and BIP, 0.4 μ M each of the outer primers F3 and B3, 6.4 U *Bst* 2.0 WarmStart DNA polymerase, and 10 ng of *wsp* standard DNA as the template. Nuclease-free (NF) water was used as the negative control. The reactions were incubated at 59–66 $^{\circ}\text{C}$ for 60 min, followed by enzyme inactivation at 80 $^{\circ}\text{C}$ for 10 min. The products were analyzed by 2% agarose gel electrophoresis, and the optimal temperature was selected for subsequent LAMP assays. Assay sensitivity was evaluated using 10-fold serial dilutions of *wsp* standard DNA prepared from a 10 ng/ μ L stock. Dilutions from 10^{-2} to 10^{-6} were tested by LAMP using incubation times of 30 and 90 min, and by PCR [7] for sensitivity comparison.

Matrix Spike Assay

To evaluate LAMP performance in different sample matrices, background DNA-containing supernatants were prepared from mosquito rearing water, five uninfected *Ae. aegypti* larvae, and five uninfected adult females using a modified crude boiling method adapted from Boza et al. [9]. Each sample matrix and control (NF water) were tested in parallel under two conditions: with or without spiked *wsp* DNA. For the spiked samples, 10 μ L of supernatant was supplemented with *wsp* standard DNA to obtain a final concentration 10-fold higher than the detection limit of the LAMP assay. All samples were used as templates in LAMP reactions with a 90-min incubation.

LAMP validation using single wAlbB-transinfected mosquitoes

The optimized LAMP assay was validated using crude DNA extracted from three individual laboratory-reared female wAlbB-transinfected Thai *Ae. aegypti* mosquitoes by the STE boiling method. For each mosquito, 1 μ L of crude DNA was added to a 9 μ L LAMP reaction master mix, yielding a 10 μ L total reaction volume, and incubated at 66 $^{\circ}\text{C}$ for 30 and 90 min.

Results:

LAMP optimization and sensitivity of LAMP assay

To optimize the LAMP reaction temperature, assays were performed at 59, 60, 62, 65, and 66°C using 10 ng/μL recombinant *wsp* plasmid DNA as the template. Agarose gel electrophoresis showed comparable amplification patterns across all temperatures (Fig. 1A). Therefore, 66°C was selected for subsequent assays. Sensitivity was evaluated using LAMP incubation times of 30 and 90 min, where 30 min represented the shortest incubation time producing a clear LAMP signal, and 90 min matched the duration of the conventional PCR assay. The 30 min reaction detected products up to the 10^{-2} dilution (Fig. 1B), whereas the 90 min reaction detected products up to the 10^{-3} dilution (Fig. 1C). Conventional PCR using primers 81F/691R detected products up to the 10^{-4} dilution (Fig. 1D).

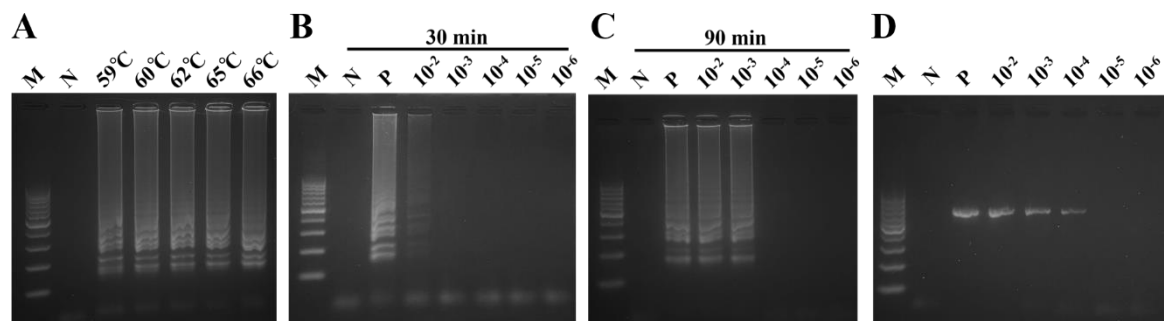


Figure 1 Optimization of the LAMP and evaluation of detection sensitivity of LAMP for *wAlbB wsp* detection. (A) Agarose gel electrophoresis of LAMP products amplified at different incubation temperatures for 60 min. (B) Agarose gel electrophoresis of LAMP products amplified at 66°C for 30 min using serially diluted *wsp* standard DNA. (C) Agarose gel electrophoresis of LAMP products amplified at 66°C for 90 min using the same dilution series. (D) Agarose gel electrophoresis of standard PCR products amplified using 35 cycles. (M = 100 bp DNA ladder, N = negative control (NF water), P = positive control (*wsp* standard DNA))

Matrix Spike Assay

The results showed that the LAMP assay of crude DNA extracts prepared from non-transfected samples successfully detected *wsp* DNA in all spiked sample matrices (Fig 2A). No amplification was observed in the corresponding non-spiked samples, indicating that the assay produced no false-positive results from non-*wsp* DNA under the tested conditions.

LAMP Validation in trans-infected mosquitoes

The optimized LAMP assay was successfully validated using DNA extracted from 3 single *wAlbB*-transfected *Ae. aegypti* mosquitoes. Agarose gel electrophoresis showed detectable amplification products after 30-min incubation (Fig. 2B), with well-resolved ladder-like patterns. These findings demonstrate that the assay is suitable for rapid and direct detection of *wAlbB* infection in individual mosquitoes under the tested conditions.

Discussion and Conclusions:

This study developed and optimized a LAMP-based assay for detecting the *wAlbB wsp* gene in *wAlbB*-transfected *Ae. aegypti*. The optimal temperature was determined to be 66°C, which enabled clear and stable amplification. Sensitivity analysis showed that LAMP detected *wsp* DNA at 100 pg/μL (2.47×10^7 copies/μL) within 30 min and 10 pg/μL (2.47×10^6 copies/μL) after 90 min, whereas conventional PCR detected 1 pg/μL (2.47×10^5 copies/μL). Although less sensitive than PCR, LAMP detected *wAlbB wsp* directly from DNA extracted from a single transfected mosquito within 30 min.

Matrix spike assays confirmed compatibility with mosquito rearing water, larvae, and adult female extracts without false-positive amplification, supporting its use for rapid individual-level screening. This preliminary LAMP assay offers a rapid and simple approach for detecting *wAlbB* infection in *Ae. aegypti*. Further validation using larger laboratory and field-collected mosquito samples, and integration with colorimetric, fluorescent, biosensor, or lateral-flow readouts, could improve its practical application in *Wolbachia* monitoring programs for arboviral disease controls.

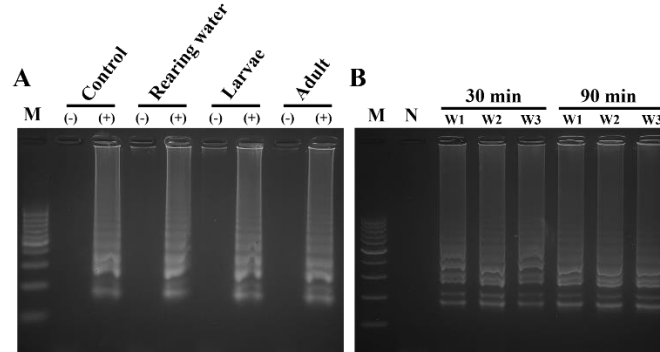


Figure 2 Matrix spike assay and single-mosquito validation of *wAlbB* LAMP detection. (A) Agarose gel electrophoresis of LAMP products from spike-in assays using different sample matrices, including control, mosquito rearing water, uninfected *Ae. aegypti* larvae, and uninfected adult females. Each matrix was tested without (-) or with (+) added *wsp* standard. (B) Agarose gel electrophoresis of LAMP products amplified from crude DNA extracted from a single *wAlbB*-transinfected *Ae. aegypti* mosquito after incubation for 30, and 90 min. M= 100 bp DNA ladder; N, negative control; W1-W3 = *wAlbB*-transinfected *Ae. aegypti* No. 1-3

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**Development of biologically
active compounds for control,
prevention and treatment of
environmental health problems
/diseases**

ANDROGRAPHOLIDE INDUCES APOPTOSIS IN PRIMARY EFFUSION LYMPHOMA (PEL) CELLS

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Abstract:

Primary effusion lymphoma (PEL) is an aggressive large B-cell non-Hodgkin lymphoma (NHL) that is typically found in immunocompromised individuals and those chronically infected with Kaposi's sarcoma-associated herpes virus (KSHV), also known as human herpesvirus 8 (HHV-8). PEL patients have a poor prognosis, with a median survival time of approximately 5–9 months and often exhibit resistance to conventional chemotherapy. Andrographolide, a bioactive diterpenoid lactone isolated from *Andrographis paniculata*, has demonstrated anticancer activity against several types of cancer. Therefore, its potential therapeutic effects against PEL were investigated in this study. The cytotoxic and pro-apoptotic effects of andrographolide were evaluated in the PEL cell line (BCBL-1). Using the MTT assay, andrographolide exhibited concentration-dependent cytotoxicity against BCBL-1 cells, with IC₅₀ values of 87.96 μ M and 37.29 μ M after 24 and 48 h of treatment, respectively. To further investigate its pro-apoptotic activity, Western blot analysis of key proteins involved in the caspase signaling pathway was performed in BCBL-1 cells treated with 50 and 100 μ M of andrographolide. The results revealed markedly increased expression of cleaved caspase-9 and poly (ADP-ribose) polymerase (PARP) in andrographolide-treated cells, indicating activation of apoptotic pathways following treatment. These findings suggest that andrographolide exerts potent cytotoxic and apoptosis-inducing effects against PEL cells and provide a foundation for future *in vivo* studies evaluating andrographolide as a potential therapeutic agent for PEL.

Keywords: Primary effusion lymphoma (PEL), Andrographolide, Anticancer, Apoptosis

Introduction:

Primary effusion lymphoma (PEL) is a rare and aggressive large B-cell non-Hodgkin lymphoma that arises in body cavities without detectable tumor masses [1] and predominantly affects immunocompromised individuals, including HIV-infected patients and organ transplant recipients [2]. PEL accounts for less than 1% of hematologic malignancies and is strongly associated with Kaposi sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), which drives lymphomagenesis through viral oncogenic mechanisms. Current treatment options, including the commonly used CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone), show limited efficacy [3], resulting in poor survival outcomes (median survival 5-9 months) and significant treatment-related toxicity. Therefore, the development of more effective and less toxic therapeutic strategies for PEL remains an urgent need.

In recent years, plant-derived natural products have gained considerable attention for the treatment of various diseases. Andrographolide, a bioactive diterpenoid lactone isolated from *Andrographis paniculata*, is a traditional herbal medicine widely used throughout Southeast Asia [4]. Previous studies have demonstrated its diverse pharmacological

properties, including anti-inflammatory, antidiabetic[5], antiviral [6], and anticancer activities. Notably, andrographolide has exhibited therapeutic potential against several malignancies, including lung cancer, hepatocellular carcinoma, leukemia, and lymphoma. Its anticancer effects are primarily mediated through the inhibition of tumor cell proliferation and the induction of apoptosis, particularly via caspase-dependent pathways involving the activation of key apoptotic proteins and subsequent programmed cell death [7].

In this study, the anticancer potential of andrographolide against PEL was evaluated with a particular focus on identifying the molecular targets and apoptotic proteins involved in caspase-dependent cell death. The findings are expected to provide mechanistic insights into the antitumor activity of andrographolide and establish a foundation for future *in vivo* investigations. Furthermore, elucidating the signaling pathways and key protein targets affected by andrographolide may facilitate the rational design of subsequent animal studies to evaluate its therapeutic efficacy, safety and potential application in PEL treatment.

Methodology:

Primary Effusion Lymphoma (BCBL-1) cells culture

Primary Effusion Lymphoma cells (BCBL-1 cell line) were maintained in RPMI-1640 medium containing 10% fetal bovine serum and 100 units/ml penicillin and 100 µg/ml streptomycin in a 5% CO₂ incubator at 37 °C.

Preparation of andrographolide stock solution

Andrographolide (Sigma-Aldrich) were dissolved in DMSO to prepare stock solutions at concentrations of 10 and 20 mM, which were stored at –20°C. Working concentrations were freshly prepared from the stock solutions prior to each experiment.

Determination of cell viability at 24 and 48 hours

BCBL-1 cells (4×10^5 cells/ml) were treated with andrographolide at varying concentrations (ranging from 0-140 µM) in a 96-well plate and were incubated in a 5% CO₂ incubator at 37 °C for 24,48 hours. After the incubation period, 5 mg/mL MTT solution (Invitrogen) was added to each well and the plates were incubated for an additional 4 hours. Following MTT incubation, a solubilizing agent (0.1 mL of 0.04 M HCl in isopropanol) was added to dissolve the formazan crystals. Subsequently, the absorbance was measured at 570 nm using a microplate reader (Tecan, Switzerland). The cell viability curves were used to calculate cell viability percentages as a following formula: [(absorbance of treated cells) / (absorbance of untreated cells)] × 100.

Protein extraction and Western blot analysis

BCBL-1 cells (4×10^5 cells/ml) were treated with different andrographolide concentrations (0, 50, and 100 µM). PEL cells were then incubated for 18 or 24 h at 37 °C in a 5% CO₂ atmosphere. They were then harvested, pelleted, and lysed in whole-cell lysis buffer in the presence of 1% protease inhibitor for 1 h on ice. The Bradford assay (Bio-Rad) was used to measure the protein concentration. Protein extracts were separated on SDS-PAGE, transferred, and blotted onto a PVDF membrane. Membranes were blocked with 1% skim milk prepared in PBS buffer, followed by incubation with the primary antibodies and then with an HRP-conjugated secondary antibody. Chemiluminescent signals were developed using Clarity Western ECL Substrate (Bio-Rad) and were detected with a Fusion FX7 Image Analyzer (Vilber, Collégien). Antibodies from Cell Signaling Technology were used for western blots including mouse monoclonal anti-cleaved caspase-9, cleaved-PARP, Bax and rabbit monoclonal anti-vinculin, as well as HRP-conjugated anti-mouse and anti-rabbit secondary antibodies.

Results and discussion:

Cytotoxicity of andrographolide on BCBL-1 cells at 24 and 48 hours

An MTT assay was performed to evaluate the cytotoxic effects of andrographolide on BCBL-1 cells. Cells were treated with various concentrations of andrographolide (0–140 μM) for 24 and 48 hours. Cell viability decreased in a concentration-dependent manner, with IC_{50} values of 87.96 μM and 37.29 μM at 24 and 48 hours, respectively (Figures 1A and 1B). In addition to BCBL-1 cells, the cytotoxic effects of andrographolide have previously been reported in other PEL cell lines, including BC-1, BC-3, GTO, and Ty-1. In BCBL-1 cells, IC_{50} values obtained in the present study were slightly higher than those reported previously, which may be attributable to differences in experimental conditions [8]. Cytopathic morphological changes were also observed in BCBL-1 cells following andrographolide treatment (Figure 2).

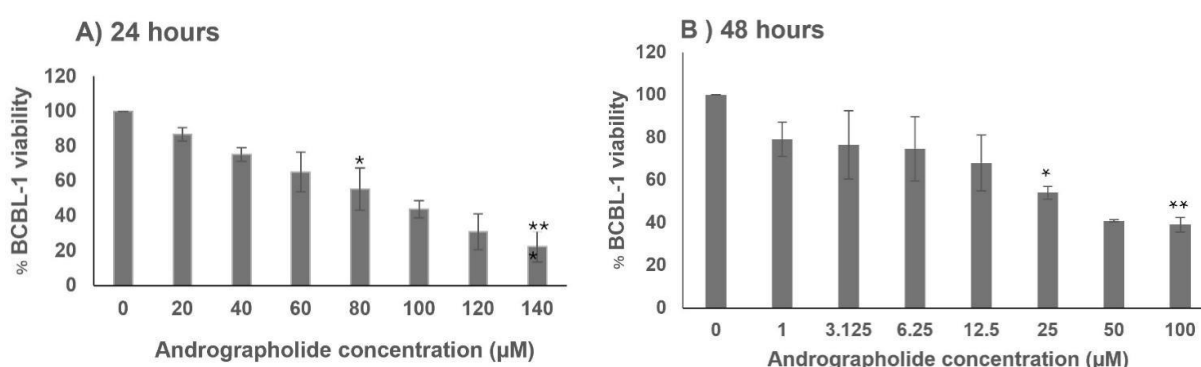


Figure 1 Cytotoxic effects of andrographolide on BCBL-1 cells. BCBL-1 cells were treated with andrographolide at concentrations ranging from 0–140 μM for 24 h (A) and 0–100 μM for 48 h (B). Cell viability was then determined using an MTT assay. Results are expressed as the mean percentage of viable cells relative to the untreated control and are presented as mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$.

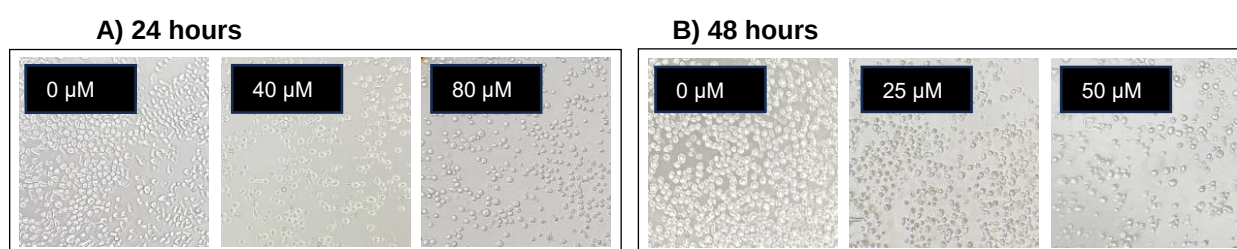


Figure 2 Cytopathic effects of andrographolide on BCBL-1 cells. BCBL-1 cells exposed to increasing concentrations of andrographolide showed concentration-dependent cytopathic morphological changes characterized by cell shrinkage and cell death. Representative images were captured using light microscopy at 200 $\times$ magnification.

Western blot analysis revealed that treatment with increasing concentrations of andrographolide (0, 50 and 100 μM) resulted in dose-dependent upregulation of cleaved PARP, cleaved caspase-9 but not BAX expression (Figure 3). Consistent with previous reports showing increased cleavage of caspase-3 and caspase-9 following prolonged andrographolide exposure [8]. These findings indicate that andrographolide promotes apoptosis in PEL cells via activation of the caspase- dependent apoptotic pathway.

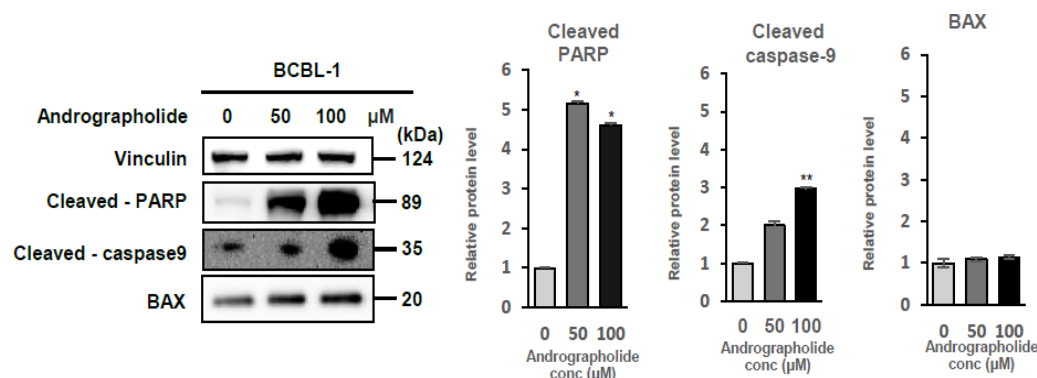


Figure 3 Andrographolide induces caspase-dependent apoptosis in BCBL-1 cells. BCBL-1 cells were treated with andrographolide (0, 50, and 100 μ M) for 18 h. Western blot analysis revealed dose-dependent upregulation of cleaved PARP and cleaved caspase-9 but not BAX. Vinculin served as the loading control.

Conclusion:

In conclusion, our findings demonstrate that andrographolide promotes cell death in BCBL-1 cells through activation of the caspase-dependent apoptotic pathway. These results provide a strong rationale for further *in vivo* studies to evaluate the efficacy of andrographolide as a potential therapeutic agent for PEL.

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ANDROGRAPHOLIDE INHIBITS CELL PROLIFERATION BY MODULATING CELL-CYCLE REGULATORS IN PRIMARY EFFUSION LYMPHOMA CELLS

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Abstract:

Primary effusion lymphoma (PEL) is an aggressive, body cavity-based B-cell lymphoma with poor prognosis and limited therapeutic options, highlighting the need for novel targeted agents. Andrographolide, a diterpenoid compound from *Andrographis paniculata*, has shown promising anti-tumor activity, but its effects on PEL remain unclear. This study investigated the impact of andrographolide on GTO cell viability and cell-cycle regulation. Treatment with increasing concentrations of andrographolide (0–140 μ M) for 24h reduced GTO cell viability in a dose-dependent manner, with an IC_{50} of approximately 74.82 μ M. Moreover, andrographolide altered the expression of key regulators involved in G1 checkpoints. Specifically, it decreased expression of CDK4 and CDK6 while upregulating p53, p21 and p16, and increased the level of phosphorylated p38 MAPK, suggesting an activation of p38–p53–CDK inhibitor signaling axis leading to G1 cell-cycle arrest. These findings indicate that andrographolide suppresses GTO cell proliferation through the modulation of cell-cycle regulatory proteins, supporting its potential as a candidate therapeutic agent for primary effusion lymphoma.

Keywords: Primary Effusion Lymphoma (PEL), Andrographolide, Cell Cycle Arrest, G1 checkpoint

Introduction:

Primary effusion lymphoma (PEL) is a rare and highly aggressive form of B-cell non-Hodgkin lymphoma, often associated with human herpesvirus 8 (HHV8) infection. Moreover, PEL primarily affects elderly or immunocompromised individuals, particularly those who are HIV-positive [1]. This cancer typically presents as malignant effusions within body cavities often without the formation of a solid tumor mass. Current treatment of PEL relies on standard chemotherapy regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone); however the prognosis remains poor for patients with PEL with a median survival of less than six months [2]. Despite current treatment options, there remains a need for more effective and less toxic therapies for PEL.

Recently, natural compounds derived from plants have increasingly gained attention as potential treatments for various cancers. Andrographolide is a diterpenoid lactone isolated from *Andrographis paniculata*, a medicinal herb widely used in traditional Asian medicine with a broad spectrum of pharmacological activities, including anticancer properties [3, 4]. The anticancer properties of andrographolide are largely mediated by the inhibition of tumor cell proliferation and the activation of caspase-dependent apoptotic pathways, leading to programmed cell death through the activation of critical apoptotic proteins [5].

This study examines the anti-proliferative effects of andrographolide on the PEL cell line (GTO) by examining its modulation of cell-cycle regulators. The findings are expected to provide insights into the anti-proliferative activity of andrographolide and contribute to future studies evaluating its potential as a therapeutic strategy for PEL.

Methodology:

Cell Culture

PEL cell line, GTO, was cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Preparation of Andrographolide Stock Solution and Treatment

Andrographolide (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions at concentrations of 20 mM, which were aliquoted and stored at -20°C. Working concentrations were freshly prepared by diluting the stock solutions in complete culture medium immediately prior to each experiment. The final DMSO concentration in culture medium did not exceed 1% (v/v). Control cells received an equivalent volume of DMSO vehicle.

Cell Viability Determination (MTT)

GTO cells (4×10^5 cells/mL) were seeded into 96-well plates and treated with andrographolide at concentrations ranging from 0 to 140 µM. The plates were incubated at 37°C in a 5% CO₂ incubator for 24 h. After treatment, MTT solution (Invitrogen) was added to achieve a final concentration of 0.5 mg/mL, and the plates were incubated for a further 4 h. The resulting formazan crystals were dissolved by adding 0.1 mL of solubilizing solution (0.04 M HCl in isopropanol) to each well, and absorbance was measured at 570 nm using a microplate reader (Tecan, Switzerland). Cell viability was expressed as a percentage of the untreated control.

Protein Extraction and Western Blot Analysis

GTO cells were treated with 0, 50, or 100 µM andrographolide for 18 h at 37°C in 5% CO₂ and then collected by centrifugation. Total protein was extracted in ice-cold RIPA buffer containing protease and phosphatase inhibitors, and quantified by Bradford assay (Bio-Rad). Equal protein amounts (30 µg) were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 1% skim milk obtained from Merck Millipore (Massachusetts, USA) in PBS, membranes were incubated overnight at 4°C with primary antibodies from Cell Signaling Technology (Massachusetts, USA) including CDK4, CDK6, p53, p21, p16, p-p38, and β-actin, followed by HRP-conjugated secondary antibodies. Signals were developed using Clarity Western ECL Substrates (Bio-Rad) and imaged with a chemiluminescence imaging system, the Fusion FX7 Image Analyzer (Vilber; Deutschland, Germany). Band intensities were quantified using ImageJ software Version 1.54p.

Results and Discussion:

Effects of Andrographolide on GTO Cell Viability

To evaluate the cytotoxic effect of andrographolide on GTO cells, an MTT assay was conducted. After the 24 h treatment, andrographolide showed a dose-dependent cytotoxic effect on GTO cells, with an IC₅₀ value of 74.82 µM (Figure 1). These results indicate that andrographolide reduces cell viability in GTO cells, suggesting a dose-dependent anti-proliferative effect that may contribute to cell death.

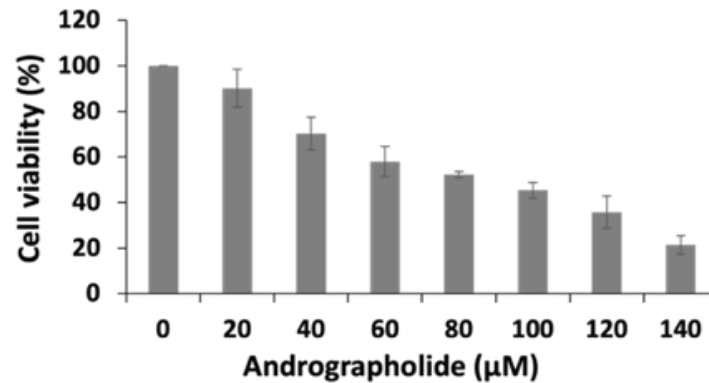


Figure 1 Effect of andrographolide on GTO cell viability. GTO cells were treated with increasing concentrations of andrographolide (0–140 μ M) for 24 h, and cell viability was assessed by MTT assay. Data are expressed as mean $\pm$ SEM .

Andrographolide Suppresses the Cell-Cycle Regulators (CDK 4 and CDK6)

To further examine the molecular mechanism related to cell proliferation, the G1/S cell-cycle regulators CDK4 and CDK6 were assessed. CDK4 and CDK6 proteins significantly decreased in a dose-dependent manner after treatment with 50 μ M and 100 μ M andrographolide compared with untreated (0 μ M) GTO cells. At the G1/S cell-cycle checkpoint, CDK4 and CDK6 form complexes with cyclin D that phosphorylate RB and drive the G1/S transition, which can lead to uncontrollable cellular proliferation [6]. Therefore, decreased CDK4 and CDK6 levels after andrographolide treatment reduce RB phosphorylation and halt G1 progression.

Andrographolide Induces p38 MAPK Activation and Upregulates p53, p21, and p16

To investigate how CDK4 and CDK6 expressions are regulated in response to andrographolide, the tumor suppressor proteins p53, p21, and p16 were analyzed. Andrographolide increased the expression levels of p53, p21, and p16 in GTO cells. Furthermore, the activation of stress-related signaling by andrographolide was examined by measuring phosphorylated p38 MAPK (p-p38). Andrographolide increased p-p38 levels at 50 and 100 μ M, indicating activation of the p38 MAPK pathway (Figure 2).

The p38 pathway can stabilize and enhance p53 activity under stress [8]. p53 is a known transcription factor that up-regulates the CDK inhibitor p21. Both p21 and p16 inhibit cyclin D/CDK4 complexes thereby preventing RB phosphorylation and blocking G1/S cell-cycle progression [7]. Collectively, the observed increase in p-p38, p53, p21, and p16, together with reduced CDK4 and CDK6, supports the idea that andrographolide activates p38 MAPK then promotes p53-driven induction of CDK-inhibitory pathways that inhibit CDK4 and CDK6, leading to G1 cell-cycle arrest.

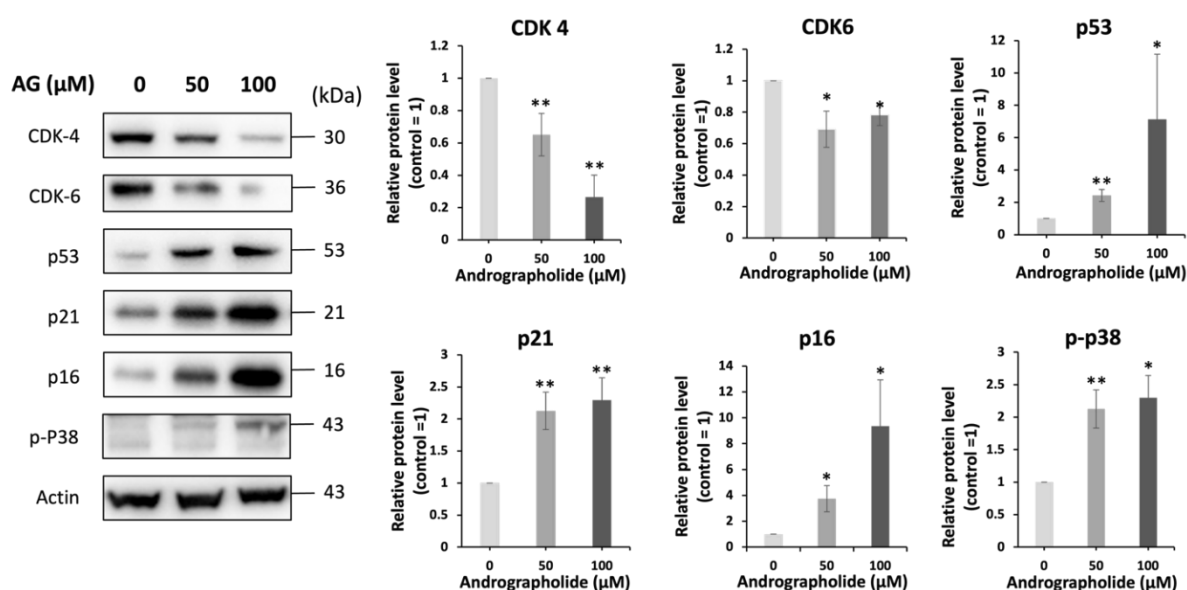


Figure 2 Effects of andrographolide on CDK4, CDK6, p53, p21, p16, and p-p38 protein expression in GTO cells. GTO cells were treated with andrographolide (0, 50, 100 μM). Bar graphs show intensity quantification of each protein relative to the untreated control (control= 1). Data represent mean $\pm$ SEM of at least three independent experiments; * $p < 0.05$ and ** $p < 0.01$ versus control.

Conclusion:

Andrographolide reduced GTO cell viability in a dose-dependent manner, with an IC_{50} of about 75 μM after 24 h treatment. Andrographolide was also found to down-regulate CDK4 and CDK6 while up-regulating p53, p21, p16, and phosphorylated p38 MAPK, indicating activation of a p38-p53-CDK inhibitor axis. Overall, these findings suggest that andrographolide induces G1/S cell-cycle arrest and thus limits the proliferation of primary effusion lymphoma GTO cells.

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CHARACTERIZATION OF BACTERIOPHAGE P1267 AGAINST MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATED FROM TROPICAL WASTEWATER

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Abstract:

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* remains a major clinical challenge, particularly in settings where antibiotic options are increasingly limited. Here, we isolated bacteriophage P1267 from tropical wastewater and examined its activity against MDR *P. aeruginosa* clinical isolates. P1267 was recovered using spot and double-layer agar assays, purified by repeated single-plaque elution, and propagated to approximately 10^9 PFU/mL. Its host range was tested against 76 MDR clinical isolates, and its biological properties were assessed by plaque morphology, one-step growth curve analysis, and thermal and pH stability assays. Virion morphology was examined by transmission electron microscopy (TEM). Among the phages tested, P1267 showed the broadest host range, lysing 48.7% (37/76) of MDR isolates. One-step growth curve analysis at an MOI of 0.01 showed a latent period of approximately 30 min and a burst size of approximately 2.5×10^3 PFU per infected cell. The phage retained infectivity from 4 to 50 °C and remained stable across pH 4-11, suggesting good tolerance to environmental variation. TEM revealed an icosahedral head of approximately 55 nm in diameter and a long, flexible, non-contractile tail measuring approximately 100 nm in length and 13 nm in width, consistent with a siphovirus morphotype. These results identify P1267 as a stable, broad-host-range lytic phage and support its further evaluation as a component of phage cocktail development against MDR *P. aeruginosa* in clinical settings in tropical regions.

Keywords: Bacteriophage, Multidrug-resistant, *Pseudomonas aeruginosa*, Siphovirus

Introduction:

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is an important opportunistic pathogen that causes pneumonia, chronic respiratory infections, etc., particularly in hospitalized and immunocompromised patients [1]. Its clinical importance is linked to an ability to resist multiple antibiotic classes. Carbapenem-resistant *P. aeruginosa* has been classified by the World Health Organization as a high-priority pathogen, underscoring the urgent need for alternative control strategies [2]. Bacteriophage therapy has regained attention as an alternative or complementary antimicrobial approach because phages are host-specific, self-replicate at the infection site, and remain active against antibiotic-resistant bacteria [3, 4].

Wastewater is a rich source for phage discovery, as it harbors dense and diverse bacterial populations including resistant strains [5]. Phages from tropical environments are exposed to elevated temperatures and variable pH, which may favor candidates with high stability. Because clinical *P. aeruginosa* isolates differ in resistance profiles and surface receptors, broad-host-range phages are valuable for therapeutic development. This study aimed to isolate and characterize bacteriophage P1267 from tropical wastewater and to evaluate its host range, replication kinetics, environmental stability, and morphology in order to assess its potential as a candidate for phage therapy against MDR *P. aeruginosa*.

Materials and Methods:

Bacterial strains and phage isolation

MDR *P. aeruginosa* hosts were grown on Tryptic Soy Agar (TSA) at 37 °C, and a single colony was cultured in Tryptic Soy Broth (TSB). Untreated wastewater was filtered (0.2 µm) and screened against the MDR host by spot test (10 µL of 10-fold dilutions spotted onto a soft-agar lawn, incubated at 37 °C overnight). Individual plaques were purified by repeated double-layer agar (DLA) elution of a single well-separated plaque to ensure clonal purity.

Propagation and titer determination

Purified phage was propagated with 10 mM CaCl₂ and log-phase host in TSB (37 °C, overnight). The lysate was centrifuged (3,200 × g, 1 h), filtered (0.2 µm), and titrated by DLA, with titer (PFU/mL) calculated as plaque count × dilution factor / plated volume (mL).

Host range, stability assays and characterization by TEM

Host range was evaluated against 76 MDR *P. aeruginosa* clinical isolates by spot assay, with the lysis rate expressed as the proportion of susceptible isolates. Plaque diameters were measured from a ruler-calibrated plate image. One-step growth curve was determined at MOI 0.01 to estimate latent period and burst size. For stability, phage (~10⁹ PFU/mL) was held for 1 h at 4, 25, 37, 50, 60, and 70 °C (thermal) or at pH 1-13 (pH); residual infectivity was assayed by DLA in triplicate (n = 3; mean ± SEM log₁₀ PFU/mL). A high-titer preparation (approximately 10⁹-10¹³ PFU/mL) was obtained by precipitating under polyethylene glycol (PEG 6000) and NaCl overnight at 4 °C, centrifuged at 16,000 rpm for 2 h at 4 °C, washed, and resuspended in SM buffer. The final titer was confirmed using DLA. For TEM, purified particles were negatively stained with 2% uranyl acetate and examined by transmission electron microscopy (TEM) at 120 kV.

Results and Discussion:

Host range against MDR P. aeruginosa

Among the phages previously screened against the 76 MDR *P. aeruginosa* clinical isolates, P1267 exhibited the broadest host range, lysing 48.7% of the isolates tested (37/76 strains). A broad host range is a desirable trait for therapeutic phages because clinical isolates vary in resistance profiles and surface receptor composition, and a single phage able to lyse many isolates reduces the need for strain-specific selection [6].

Plaque morphology and replication kinetics

P1267 produced clear, round plaques, each with a dense core surrounded by a translucent halo (Figure 1A-C). Plaque diameters, measured from a ruler-calibrated plate image, ranged from 0.98 to 2.71 mm (mean 1.80 ± 0.46 mm, n = 23; Figure 1D). One-step growth analysis at an MOI of 0.01 indicated a latent period of approximately 30 min followed by a sharp rise in titer, giving a mean burst size of approximately 2.5 × 10³ PFU per infected

cell (Figure 1E). This combination of a short latent period and large burst size reflects efficient intracellular replication and rapid progeny release, traits that favor fast clearance of the host and make P1267 attractive for therapeutic application against MDR *P. aeruginosa* [7].

Thermal and pH stability

P1267 remained fully infective from 4 to 50 °C, with a marked decline at 60 °C and substantial loss at 70 °C (Figure 1F). It was stable across pH 4-11, with optimal infectivity at pH 5-9, while activity was lost under strongly acidic (pH 1) and alkaline (pH ≥ 12) conditions (Figure 1G). This broad temperature and pH tolerance is advantageous for storage, handling, and field application, and is consistent with adaptation to the fluctuating thermal and pH conditions of the tropical wastewater from which the phage was recovered [8].

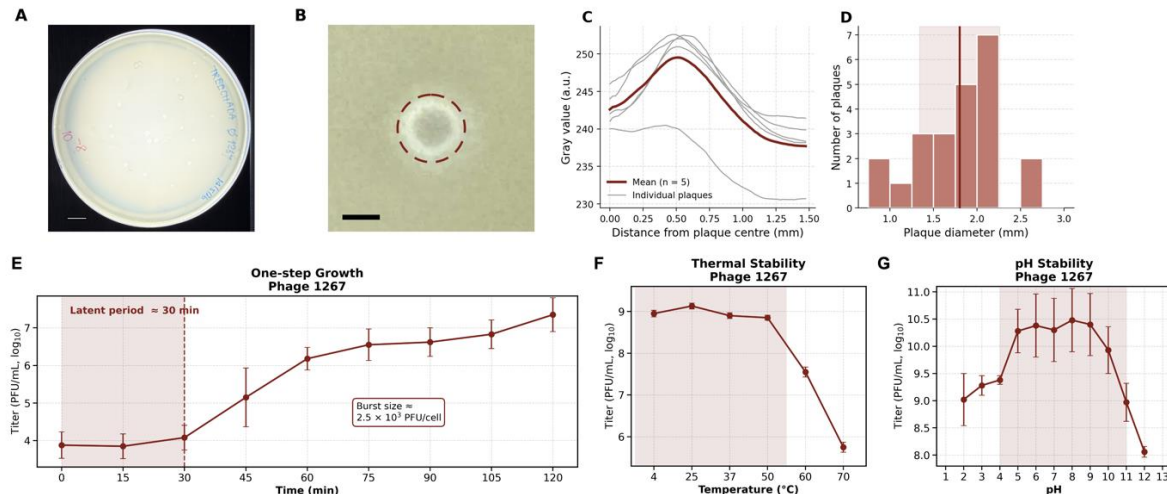


Figure 1 Plaque morphology of phage P1267 on its MDR *P. aeruginosa* host. (A) Whole-plate overview; scale bar, 10 mm. (B) Representative plaque showing a dense core surrounded by a translucent halo; the dashed circle marks the plaque margin (scale bar, 1 mm, approximate). (C) Radial gray-value intensity profiles of five individual plaques (gray) with the mean (dark red), showing the bright halo ring surrounding the core. (D) Plaque size distribution; mean diameter 1.80 ± 0.46 mm (SD), $n = 23$, range 0.98 to 2.71 mm. (E) One-step growth curve at a multiplicity of infection of 0.01, indicating a latent period of approximately 30 min (shaded) and a burst size of approximately 2.5×10^3 PFU per infected cell; points are the geometric mean of three replicates with error bars of ± 1 SD ($\log_{10}$ scale). (F) Thermal and (G) pH stability profiles; shaded areas indicate the stable zone, and data are mean $\pm$ SEM ($n = 3$).

Virion morphology

TEM of negatively stained particles revealed virions with an icosahedral head approximately 55 nm in diameter and a long, flexible, non-contractile tail approximately 100 nm in length and 13 nm in width (Figure 2A-C), consistent with the siphovirus morphotype within the class *Caudoviricetes* [9]. This structural classification, together with the broad host range and environmental stability described above, supports the potential of P1267 as a therapeutic candidate, although genomic analysis remains necessary to confirm the absence of toxin, antibiotic-resistance, and lysogeny-associated genes before therapeutic development.

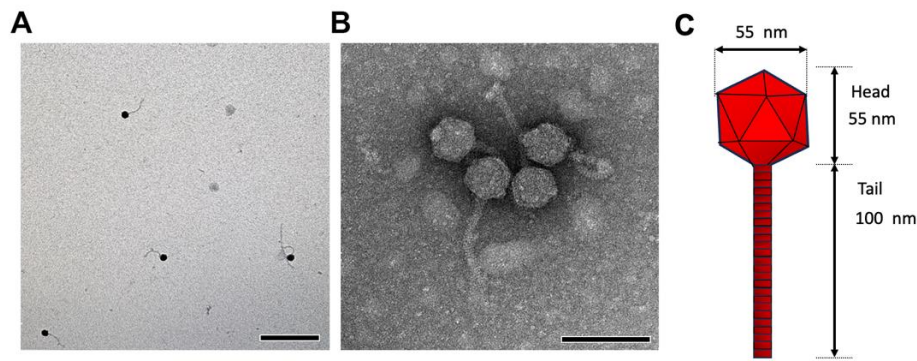


Figure 2 Transmission electron micrographs and schematic representation of phage P1267 (A) Low-magnification overview showing dispersed virions across the field of view; the inset shows a single intact particle with an icosahedral head and a long tail (scale bar, 200 nm). (B) Higher-magnification field showing a cluster of virions, each with an icosahedral head and a long, non-contractile tail (scale bar, 100 nm). (C) Schematic rendition of the P1267 virion summarizing its dimensions.

Conclusion:

Bacteriophage P1267, isolated from tropical wastewater, is a broad-host-range lytic phage that lysed 48.7% (37/76) of MDR *P. aeruginosa* isolates, replicated efficiently, and remained stable across 4-50 °C and pH 4-11 with a siphovirus morphotype. These properties support P1267 as a promising candidate for further development as an antimicrobial agent against MDR *P. aeruginosa*.

Acknowledgements

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DEVELOPMENT OF DROPLET DIGITAL POLYMERASE CHAIN REACTION (ddPCR) FOR DETECTION OF *Legionella*

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Abstract:

Legionnaires' disease has emerged as a critical public health concern, with *Legionella pneumophila* recognized as the predominant cause of waterborne outbreaks worldwide. Conventional culture methods remain the standard for diagnosis, but they are time-consuming, requiring approximately two weeks of incubation, and may fail to detect bacteria present at low concentrations or in a viable but non-culturable state. To overcome these limitations, this study developed and optimized a droplet digital polymerase chain reaction (ddPCR) assay targeting the macrophage infectivity potentiator (*mip*) gene. The optimization assays identified 54 °C as the optimal annealing/extension temperature and 900/250 nM as the most effective primer/probe concentration, the assay demonstrated clear droplet separation and reproducibility. The limit of blank (LOB) was determined to be 0.13 copies/μL. Analytical sensitivity testing demonstrated reliable detection at 1 pg/μL, with copy numbers consistently above the LOB, and the limit of detection (LOD) was calculated as 0.348 copies/μL. Analytical specificity was confirmed by testing genomic DNA from fourteen waterborne bacterial species, where positive droplets were observed specifically in *L. pneumophila* DNA, with no cross-reactivity detected in non-target waterborne organisms. The ddPCR assay is well suited for applications in environmental monitoring and water safety surveillance, and it support quantitative microbial risk assessment by enabling accurate detection of low-level contamination.

Keywords: *Legionella pneumophila*, Legionnaires' disease, Droplet digital polymerase chain reaction, Macrophage infectivity potentiator (*mip*), Water safety monitoring

Introduction:

Legionnaires' disease has become a major public health issue, with *L. pneumophila* recognized as the predominant cause of waterborne outbreaks globally. The disease typically presents with pneumonia-like symptoms, including high fever, cough, shortness of breath, muscle aches, and gastrointestinal manifestations such as diarrhea, which can progress to severe pneumonia and respiratory failure. The organism is commonly associated with aquatic environments, and exposure to contaminated water can lead to infection (1). In Thailand, travel-associated cases have been increasingly reported among tourists, emphasizing the need for strengthened surveillance and water safety management (2). Conventional detection approaches, in which *Legionella* spp. are cultured, are labor-intensive and require a prolonged incubation period of about two weeks and may be limited when bacterial concentrations are low or when *L. pneumophila* exists in a viable but non-culturable state. Droplet digital polymerase chain reaction (ddPCR) offers several advantages over conventional amplification-based methods, including absolute quantification without standard curves, improved sensitivity, high precision, strong performance at low target concentrations, and enhanced tolerance to PCR inhibitors (3). These features make ddPCR particularly useful for water testing and quantitative microbial risk assessment (4). The macrophage infectivity

potentiator (*mip*) gene is a well-established target for *L. pneumophila* detection because of its specificity and suitability for molecular diagnosis. In this study, a ddPCR assays targeting *mip* gene was developed and optimized, according to MIQE and dMIQE2020 guidelines (5) to improve analytical sensitivity, specificity, and quantitative accuracy for environmental applications.

Methodology:

Bacteria cultured and DNA extraction: *L. pneumophila* and other thirteen waterborne bacterial species were cultured for specificity testing, comprising four Gram-positive strains and nine Gram-negative strains. Each organism, except *L. pneumophila*, was cultured on LA agar and incubated at 37°C for 18 hours. While *L. pneumophila* was cultured on BCYE agar and incubated at 35°C for 2 weeks. Genomic DNA from Gram-positive strains was extracted using a commercial kit according to the manufacturer's instructions. Genomic DNA from Gram-negative strains was extracted using a modified ethanol precipitation protocol.

ddPCR assay design and optimization: A ten-fold serial dilution series of the *L. pneumophila* genomic DNA was used to determine the optimal dilution range and to optimize annealing/extension temperature and primer/probe concentration. Several reaction conditions were evaluated to identify the setting that produced the clearest droplet separation and the most stable fluorescence amplitude.

Limit of blank determination: To define the analytical limitation of the ddPCR assay, the limit of blank (LOB) was determined as an indicator of assay performance, specifically its capacity to distinguish true negatives from background noise, and was calculated by measuring the results of the blank replicates and computing according to the following equation: $LOB = \text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}})$ (6).

Analytical sensitivity and limit of detection: Ten-fold serial dilution of the *L. pneumophila* genomic DNA were tested to determine sensitivity of the ddPCR, from 10 ng/μL (UN) down to a theoretical dilution of 1 ag/μL (10^{-10}). The limit of detection (LOD) was defined as the lowest concentration consistently detected above the LOB in at least 95% of replicates. The resulting data were used to determine the sensitivity threshold of the assay and to evaluate its capability for detecting low-abundance targets. The LOD can be express as: $LOD = LOB + 1.645(\text{SD}_{\text{lowest concentration samples}})$ (6).

Analytical specificity: Genomic DNA from all bacterial isolates was tested under optimized ddPCR conditions. Fluorescence signals were analyzed to determine amplification outcomes. A reaction was considered specific if positive droplets are observed exclusively in the *L. pneumophila* genomic DNA sample, with all non-target bacterial DNA yielding no detectable amplification or remaining below the manually defined fluorescence threshold. This assessment confirmed the assay's specificity and its capacity to differentiate *L. pneumophila* from phylogenetically related and unrelated bacterial species.

Results and Discussion:

Annealing/Extension temperature and primer/probe concentration effects on ddPCR performance for *mip* gene detection in *L. pneumophila*.

Fluorescence amplitude plots across a gradient of temperatures revealed that 54°C provided the clearest separation between positive and negative droplets, minimizing background noise and ensuring stable threshold definition (Fig 1A). Among the tested specific primer/probe concentrations, 900/250 nM provided the clearest separation between positive and negative droplets with stable amplitude. These conditions were selected as

optimal, in accordance with MIQE/dMIQE2020 guidelines emphasizing droplet resolution and reproducibility (Fig 1B) (5). The optimized conditions were selected for subsequent LOB, sensitivity, specificity, and LOD determination. LOB was determined to be 0.13 copies/ μ L under the optimized ddPCR conditions.

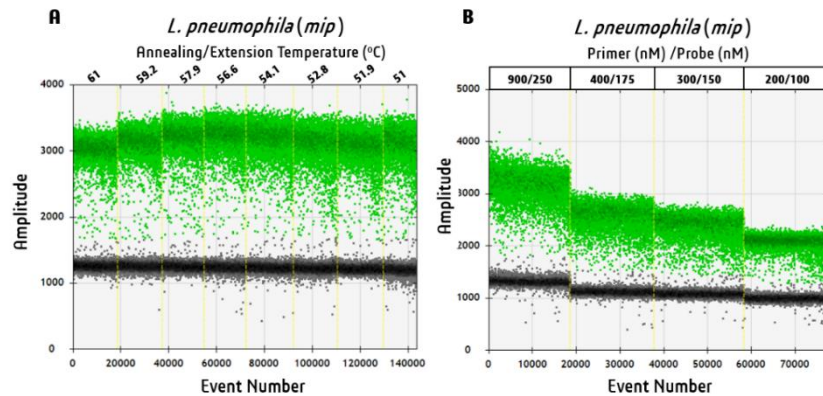


Figure 1 Annealing temperature and primer/probe concentration influence on droplet separation in ddPCR targeting *mip* gene. (A) Droplet separation at different annealing/extension temperatures between 51°C to 61°C. (B) Droplet separation at varying primer/probe concentrations including 900/250, 400/175, 300/150, 200/100 nM, respectively.

Analytical sensitivity and limit of detection of ddPCR assay for *mip* gene in *L. pneumophila*

Analytical sensitivity testing demonstrated that the ddPCR assay could reliably detect *mip* gene at concentrations as low as 1 pg/ μ L (10^{-4}). At this level, the measured copy number remained above the LOB (0.13 copies/ μ L), representing the lowest concentration at which positive droplets can be distinguished from background noise (Fig 2). Based on replicate analysis and statistical calculation, LOD was determined to be 0.348 copies/ μ L. This value confirms that the optimized ddPCR assay is capable of detecting minimal target quantities with high sensitivity.

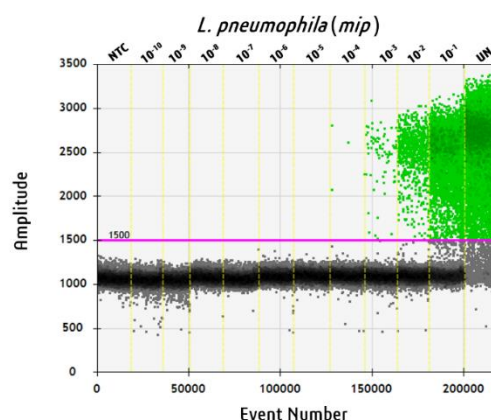


Figure 2 ddPCR performance across ten-fold dilution series for *mip* gene detection in *L. pneumophila*. The lowest concentration tested, 1 pg/ μ L (10^{-4}), produced copy numbers above the limit of blank (0.13 copies/ μ L), indicating the minimum detectable level of the assay. The threshold was set at 1,500 HEX amplitude. NTC: No-template control.

Analytical specificity of ddPCR assay using *mip*-specific primer and probe against waterborne pathogens

Analytical specificity of the ddPCR assay was ensured through the use of primers and probe sequences specifically designed for the *mip* gene of *L. pneumophila*. Positive droplets were consistently observed only in *L. pneumophila* DNA, whereas all non-target bacterial DNA produced no amplification above the threshold. These results confirm that the specific primer/probe set selectively amplifies the *mip* gene without cross-reactivity, validating the assay is high analytical specificity.

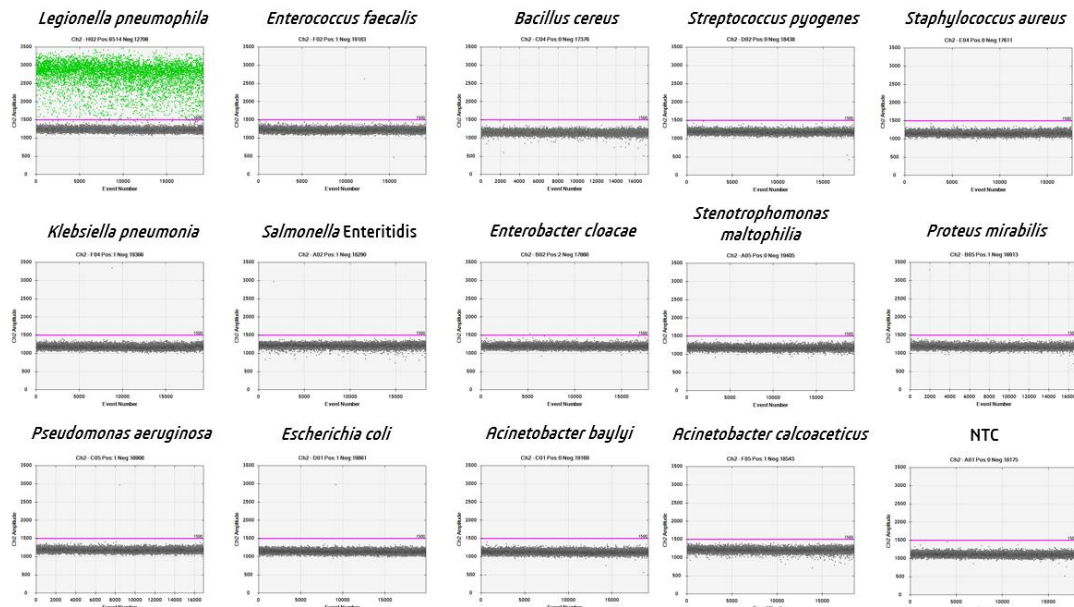


Figure 3 ddPCR specificity testing using *mip*-specific primers and probe of *L. pneumophila* against thirteen waterborne pathogens. Positive droplets were detected exclusively in *L. pneumophila* DNA, while all non-target bacterial species and the no-template control showed only negative droplets below the threshold, confirming the absence of cross-reactivity. The threshold was set at 1,500 HEX amplitude. NTC: No-template control.

Conclusion:

The ddPCR assay targeting the *mip* gene of *L. pneumophila* was optimized to provide clear droplet separation and reproducibility. It demonstrated reliable detection at low concentrations and showed exclusive amplification of *L. pneumophila* without cross-reactivity. The assay therefore offers high sensitivity and specificity for water safety monitoring.

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DIGITAL COLORIMETRIC ANALYSIS FOR EVALUATING CADMIUM UPTAKE IN RICE ROOT TISSUE

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Abstract:

Evaluating heavy metal accumulation in agronomic crops is essential for ensuring food safety. However, conventional analytical techniques are often time-consuming, labor-intensive, and costly, limiting their application in large-scale crop breeding programs that require rapid phenotypic screening. To address this challenge, this study evaluated a digital colorimetric method as a rapid, cost-effective alternative for assessing cadmium (Cd) accumulation in rice roots. Rice seedlings were grown hydroponically under Cd concentrations ranging from 0 to 20 mg L⁻¹ for 14 days. Three representative rice cultivars (IR64, PSL2, and KDML105) were initially used to validate the method, followed by an expanded evaluation of five major cultivars (PSL2, KDML105, Suphan1, RD31, and Chorati) for comprehensive phenotypic screening. Following harvest, root systems were subjected to histochemical staining, and the proportion of red-stained root area was quantified using digital image analysis. The colorimetric method successfully distinguished cultivars with high and low Cd accumulation. Method validation demonstrated a strong positive correlation between image-derived root traits and manually measured root biomass ($R^2 = 0.8659$). Furthermore, the percentage of red-stained root area showed a strong linear relationship with Cd concentrations in root tissues determined by flame atomic absorption spectrometry (FAAS) ($R^2 = 0.911$). These results demonstrate that digital colorimetric analysis provides a rapid, reliable, and low-cost approach for large-scale phenotypic screening of cadmium accumulation in rice roots, offering a practical tool for breeding programs aimed at developing rice cultivars with reduced heavy metal accumulation.

Keywords: Cadmium, Colorimetric analysis, Heavy metal, Rice, Stain

Introduction:

Cadmium (Cd) contamination in agricultural soils, particularly in Mae Sot, Tak Province, Thailand, poses a severe threat to food safety and human health. Rice (*Oryza sativa* L.) efficiently absorbs and translocates Cd from roots to grains. This makes it a primary dietary vector for human renal dysfunction, bone degradation, and cardiovascular diseases. High internal Cd accumulation impairs plant growth, causing chlorosis, membrane damage, and severe root stunting that limits nutrient acquisition. While breeding low-accumulating varieties can mitigate these risks, traditional screening workflows utilizing Flame Atomic Absorption Spectrophotometry (FAAS) or Inductively Coupled Plasma (ICP) spectroscopy create severe operational bottlenecks due to destructive, labor-intensive mineral acid digestions and multi-day drying phases (Sriprachote et al., 2012).

Furthermore, internal Cd accumulation and distribution within root tissues vary significantly depending on genotypic variations in heavy metal tolerance. Consequently, developing a rapid methodology to screen these diverse tissue-specific accumulation patterns is essential for accelerating low-accumulating crop breeding. To overcome these constraints, this study establishes a low-cost colorimetric imaging framework using histochemical

chelating agents to map spatial Cd concentrations in root architectures. The method was validated across selected rice varieties to optimize rapid, non-destructive phenotypic selection traits at early developmental stages.

Methodology:

Plant Materials and Hydroponic Cultivation

Six rice varieties including: IR64, PSL2, KDML105, Suphan1, RD31, and Chorati were used in this study. Seeds were surface-sterilized and germinated in darkness for 7 days and exposed to light for 3 days. Seedlings were acclimated in 25% Yoshida solution for 7 days before transfer to a 50% baseline solution (pH 5.5). Following a baseline optimization shift in the experimental scope, IR64 was excluded. The remaining varieties from both groups were combined into a final 5-variety set (PSL2, KDML105, Suphan1, RD31, and Chorati) for a continuous 14-day Cd stress gradient (0, 5, 10, 15, and 20 mg L⁻¹) prepared from a 1,000 ppm CdCl₂ stock solution.

Histochemical Staining and Image Analysis

Harvested root networks were incubated for exactly 30 min in the chelating alkaline solution to form an insoluble red Cd-coordination complex. Rinsed and blotted roots were digitized using an Epson V800 scanner and spatially calibrated against a stage micrometer in ImageJ. Using the HSB color threshold function, two pixel segmentation metrics were extracted: total root surface area (cm²) to index structural toxicity, and the percentage of red area (% Red Area) relative to the total footprint to quantify localized tissue Cd sequestration. The background pixels were digitally subtracted to prevent confounding artifacts, ensuring that only the specific red-stained chromatic regions were isolated for statistical evaluation (Figure 1).

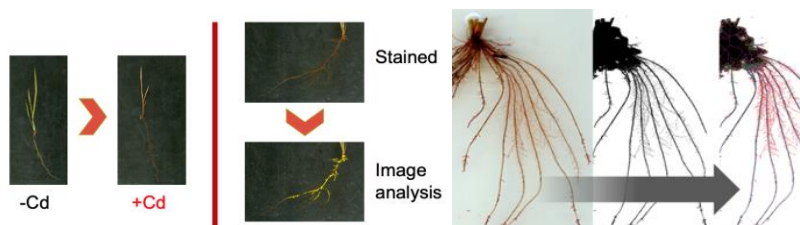


Figure 1 Stained rice seedling and selected area for image analysis.

Biomass Validation and Chemical Quantification

Imaged roots were oven-dried at 60°C for 3 days to record true dry weights (g). For chemical validation, 0.5 g of ground root tissue was digested in a 2:1 HNO₃:HClO₄ matrix on a stepped block digester (100°C for 30 min, 150°C to yellow slurry, and 200°C to clarity), followed by boiling with HCl at 160°C. Digested fractions were filtered, diluted to 25 mL with 1% HNO₃, and analyzed for absolute Cd concentration via analytical FAAS. Analytical accuracy was monitored throughout the quantification process using blank samples and certified reference materials to ensure rigorous baseline calibration.

Statistical analysis

Datasets were analyzed in R (v3.5) using One-Way ANOVA and Tukey's HSD post-hoc test ($P < 0.05$). Continuous characteristics were cross-validated via linear regression models (R^2), while population-wide colorimetric trends across the treatment gradient were mapped using second-order non-linear polynomial curves.

Results, Discussion and Conclusion:

Effects of cadmium on root growth and structural stunting

Cadmium adversely affected plant growth and development. Under peak stress conditions ($20 \text{ mg L}^{-1} \text{ Cd}$), severe root stunting occurred population-wide, though individual cultivars displayed distinct phenotypic plasticity. Relative root area reductions reached 83.56% for PSL2, 62.95% for KDML105, 83.80% for Suphan1, 82.78% for RD31, and 76.71% for Chorati. Cultivars maintaining greater architectural stability, such as KDML105, demonstrated higher morphological tolerance. Conversely, highly susceptible cultivars like Suphan1, PSL2, and Chorati suffered rapid shifts across significance categories, culminating in root elongation arrest and sudden biomass collapse. This structural stunting is largely attributed to Cd-induced inhibition of root cellular elongation and the disruption of essential nutrient translocations. These cellular impairments ultimately trigger premature senescence and architectural degradation within the localized root tissues.

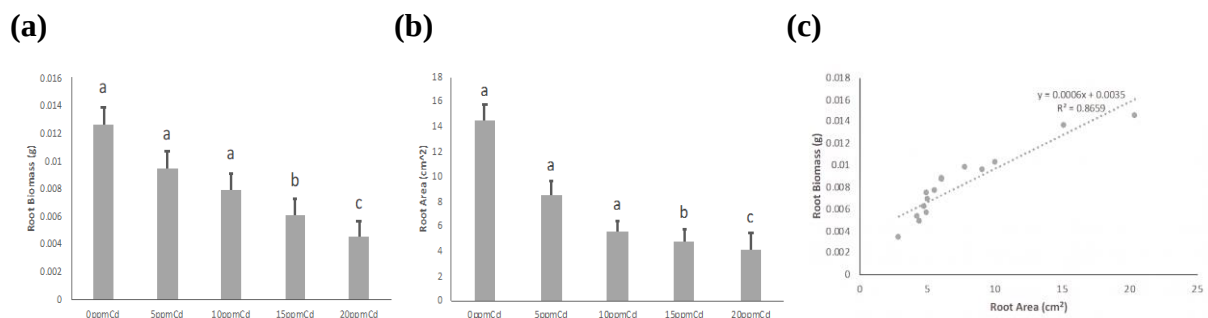


Figure 2 Effect of Cd on growth metrics for the average of five rice varieties: (a) root biomass under different Cd concentrations; (b) root area under different Cd concentrations; and (c) linear correlation between digital root area and manual dry biomass ($R^2 = 0.8659$).

Methodological validation of digital image tracking

To validate this non-destructive tracking method, image-derived surface metrics were regressed against standard analytical measurements. Continuous regression modeling confirmed a strong, significant positive linear correlation ($R^2 = 0.8659$) between digital root surface area and manual root dry biomass, proving that automated color segmentation precisely replicates physical structural characteristics across the multi-variety population (Figure 2c). Consequently, digital surface area estimations can effectively substitute for destructive manual weighing, eliminating labor-intensive, multi-day drying bottlenecks during large-scale genotypic screenings.

Colorimetric quantification and elemental mass correlations

Colorimetric pixel quantifications differentiated core genotypic strategies: KDML105 developed a significantly greater % Red Area than PSL2 at identical treatment levels. The minimal coloration density and distinctive statistical grouping of PSL2 align with a high-efficiency metal excluder mechanism that restricts root entry or sequesters toxic ions

defensively to outer cellular spaces. In contrast, the heavy red pixel deposition in KDML105 defines it as a high accumulator genotype (Figure 3c). Methodological validation established a positive linear relationship ($R^2 = 0.911$) between image-estimated root % Red Area and true internal Cd tissue concentrations quantified through FAAS (Figure 3a), confirming that pixel-based color density scales predictably with chemical tissue content. When evaluating population-wide dynamics against expanding exposure levels, the average root area model tracked negatively and linearly against external solutions ($R^2 = 0.940$), while the average root % Red Area conformed to a stable second-order non-linear polynomial curve ($R^2 = 0.977$) (Figure 3b). Our results confirm the capability of the developed colorimetric framework to classify phenotypic traits from low to high Cd accumulators.

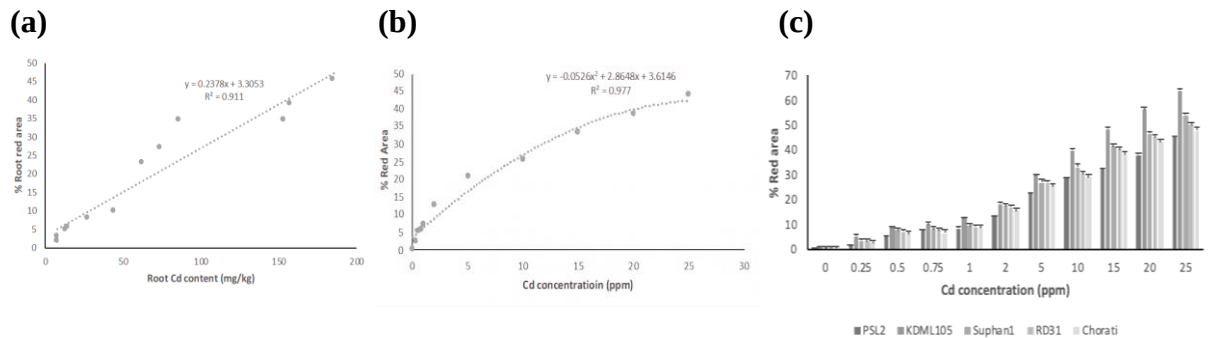


Figure 3 Computational colorimetric analysis of five rice varieties: (a) linear correlation between root % red area and true internal tissue Cd content ($R^2 = 0.911$); (b) second-order non-linear polynomial curve mapping population average of % red area against external solution Cd concentrations ($R^2 = 0.977$); and (c) genotypic distribution of % red area across individual cultivars under expanding solution exposure levels.

Conclusion:

This study validated an integrated high-throughput digital colorimetric methodology to evaluate cadmium toxicity and localization in rice root networks. By substituting traditional spectroscopic bottlenecks with rapid image thresholding and histochemical chelation, this cost-effective framework reliably indexes physical biomass reductions and mathematically tracks internal metal accumulation. Ultimately, this approach enables rapid, non-destructive genotypic classification and high-throughput phenotypic screening of heavy metal distribution for large-scale agricultural breeding applications.

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**Safety and toxicity of food,
drugs and other chemicals
including biofuels**

MECHANISTIC INVESTIGATION OF THE ANDROGENIC ENDOCRINE-DISRUPTING EFFECTS OF EMERGING ENVIRONMENTAL CONTAMINANT POLYHALOGENATED CARBAZOLES (PHCZs)

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Abstract:

Polyhalogenated carbazole (PHCZs) are emerging contaminated chemicals, which have been detected in many organisms, soil, water and housedust. PHCZs can produce naturally and generated through anthropogenic activities such as production of indigo dye, photoelectric material intermediate, phosphorescent, and electroluminescent devices. Apart from environmental detections, humans may be exposed to PHCZs through multiple routes. The detection of PHCZs in human urine confirms that humans have been exposed to PHCZs. However, information regarding endocrine disrupting effect of PHCZs remains limited, especially its impact on androgen receptor (AR) activity. Therefore, six PHCZs were tested for their endocrine disrupting potential in vitro assay. AR luciferase reporter gene assay was used to evaluate agonist or antagonist activity in MDA-kb2 cell lines. Cells were treated under two conditions: in the presence of testosterone and in the absence of testosterone at different concentrations of PHCZs ranging from 0.01 μ M to 20 μ M. The result showed that five PHCZs significantly decreased luciferase activity, indicating that five PHCZs exhibited significant anti-androgenic activity on AR. We further evaluated mechanisms of AR inhibition by evaluating dose-response curve shifts and slope characteristics. The result demonstrated a rightward parallel shift in the dose response curves without significantly affecting the maximal response, highlighting that PHCZs act as competitive AR inhibitors targeting the ligand-binding domain. The effect of PHCZs on AR may lead to adverse effects on development and the reproductive system in humans and wildlife, resulting in negative impacts on ecosystem and human health.

Keywords: Polyhalogenated carbazole, Endocrine disrupting chemical, Androgen receptor, Anti-androgen, Competitive inhibitor

Introduction

Industrial and technological advancements aimed to meet modern demands have resulted in widespread chemical pollutions. Various chemicals used as raw materials or generated as products and daily consumer goods, can be released into environment. While some chemicals persist in the environment and negatively impact human health. Endocrine disrupting chemicals (EDCs) are environmental pollutants that represent a major public health and environmental concerns. EDCs interfere with critical biological processes (Yilmaz et al., 2020). They can also interfere with the male reproductive system. This interference can cause disruption in hormonal functions, urogenital congenital anomalies, reduce sperm quality and sperm count, resulting in infertility. World Health Organization (WHO) report in 2021 stated that one in six people worldwide experience infertility (World Health Organization, 2023).

Polyhalogenated carbazoles (PHCZs) are the emerging contaminated chemicals, which was first discovered in 1984 in Buffalo River, USA and have been increasingly detected in Canada, Germany, and China. PHCZs are halogenated organic compounds that have been substituted by chlorine (Cl), bromine (Br), and iodine (I), in the carbazole structure (Ji et al., 2021). PHCZs have been detected in many organisms and environmental media including soil, sediment, water, and atmospheric air. PHCZs can produce naturally through microorganisms, wildfires and volcanic eruptions. Other PHCZ sources are generated through anthropogenic activities. Due to industrial and technological advancements, human activities are expected to become the primary source of PHCZ emissions in the future (Tu et al., 2025).

The detection of PHCZs in human urine has raised significant concerns regarding potential health risks (Mao et al., 2024). From previous study have been indicated exposure to PHCZs show adverse effect on cardiac and hepatic functions. (Du et al., 2022; Ji et al., 2023; Wang et al., 2025). Furthermore, PHCZs can disrupt endocrine systems by inhibiting glucocorticoid receptor (GR) and activating estrogen receptor (ER) in vivo models. The study shows that PHCZs were found to increase epithelial and uterine weight in rats (Yue et al., 2020). However, to date, no studies have systematically characterized the biological and toxicological effects of PHCZs on AR signaling. Therefore, in this study we aim to investigate the effect of PHCZs on AR.

Methodology:

Cell viability assay

The cytotoxicity of PHCZs was evaluated by a colorimetric MTT assay in human breast cancer cell line (MDA-kb2), which measures the reduction of tetrazolium salt into insoluble formazan crystals product by the mitochondria. The cells were seeded in the clear 96-well microtiter plated at 3.0×10^4 cells/well. Cells were treated with PHCZs at different concentrations ranging from 0.01 μ M to 20 μ M or OHF at different concentrations ranged from 0.01 nM to 5 μ M (as a positive control) or DMSO (as vehicle control) to examine androgenic activity, and these cells were co-treated with testosterone at a concentration of 625 pM (at EC₅₀ concentration) and PHCZs or at different concentrations and DMSO to examine anti-androgenic activity. Formazan color intensity was measured by microplate reader, which absorbance at 570 nm with reference wavelength of 650 nm. To evaluate cytotoxicity that can occur to the cell.

Androgen receptor (AR)-mediated luciferase reporter gene assay

To assess the activity of PHCZs on AR, MDA-kb 2 cells were seeded in white 96-well microtiter plates at a density of 3.0×10^4 cells/well (Tachachartvanich et al., 2022). This Cells were transfected with an androgen-responsive luciferase reporter plasmid regulated by the MMTV promoter. Cells were treated with different concentrations of PHCZs ranged from 0.01 μ M to 20 μ M or OHF at different concentrations ranged from 0.01 nM to 5 μ M (as a positive control) or DMSO (as vehicle control) in the absence of testosterone to evaluate potential androgenic activity. Assessment of potential anti-androgenic activity, MDA-kb 2 cells were co-cultured with 625 pM testosterone (EC₅₀) and PHCZs or OHF. Cells were incubated for 18 h and then measurement luciferase activity by micro plate reader.

Mechanism of androgen receptor (AR) inhibition elicited by PHCZs

To evaluate the mechanism of AR inhibition, MDA-kb 2 cells were co-cultured with different concentrations of testosterone ranged from 19.6 pM to 20,000 pM with non-cytotoxicity concentration of each PHCZs at 1 μ M and 20 μ M or 0.05 μ M OHF as the positive control or DMSO as a vehicle control. Cells were incubated for 18 h and then measurement luciferase activity by using micro plate reader.

Results and Discussion:

MTT assay was performed to evaluate cytotoxicity, confirming that the observed effects were not confounded by cell toxicity. The concentrations of PHCZs tested showed no significant cytotoxicity. The endocrine-disrupting potential of six PHCZs (3-B-4,9-DCCZ, 6-B-9-PCZ, 3-B-6-PCZ, 6-BCZ, 6-PCZ and 2,2-CCZ) on the AR was assessed using an AR-mediated luciferase reporter gene assay. Five PHCZs (6-B-9-PCZ, 3-B-6-PCZ, 6-BCZ, 6-PCZ, and 2,2-CCZ) significantly reduced luciferase activity in MDA-kb2 cells, indicating anti-androgenic activity. While, 3-B-4,9-DCCZ significantly increased luciferase activity at higher concentrations when compared to the testosterone alone. The activity of 3-B-4,9-DCCZ was similar to that of hydroxyflutamide (OHF), which is a known partial antiandrogen by increasing luciferase activity at higher concentrations in the low concentrations of testosterone. Among the PHCZs tested, 6-PCZ exhibited strongest anti-androgenic effect, followed by 6-BCZ, 2,2-CCZ, 3-B-6 PCZ, and 6-B9-PCZ respectively. On the other hand, six PHCZs did not show significant androgen activity.

Five PHCZs were further examined the mechanism of AR inhibition by evaluating the shift of dose-response curves and slope characteristics. The result demonstrated that all five PHCZs produced a parallel rightward shift in the dose-response curves without significantly affecting the maximum response, similar to the effect observed with the positive control, OHF. This pattern is consistent with competitive receptor antagonism, suggesting that the PHCZs act as competitive inhibitors of AR signaling.

The effects of PHCZs on AR are similar to that of the per- and poly- fluoroalkyl substances (PFASs) (Tachachartvanich et al., 2022) and bisphenol A (BPA) (Teng et al., 2013), which can also act as anti-androgens by competing with AR. Previous studies have reported that increasing BPA concentrations in urine are associated with reduced sperm concentration, sperm count, sperm motility, and sperm vitality (Li et al., 2011). BPA may also disrupt spermatogenesis. Studies have reported that exposure to BPA during pregnancy can affect fertility. Therefore, PHCZs may alter sexual development and reproductive function, potentially affecting human health.

Conclusion:

This study is the first to report the endocrine-disrupting effects of PHCZs on the AR. The result demonstrated that five PHCZs (6-B-9-PCZ, 3-B-6-PCZ, 6-BCZ, 6-PCZ, and 2,2-CCZ) exhibited antagonistic activity toward AR. The mechanism AR inhibition of PHCZs shown that they are competitive AR inhibitor. Among the tested compounds, 6-PCZ exhibited the strongest AR antagonistic activity. Therefore, exposure to PHCZs may disrupt AR signaling pathways, potentially leading to adverse effects on sexual development, reproductive system, and fertility.

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MECHANISTIC INVESTIGATION OF THE ANDROGENIC ENDOCRINE-DISRUPTING EFFECTS OF EMERGING ORGANOPHOSPHATE FLAME-RETARDANTS

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Abstract:

Organophosphate flame-retardants (OPFRs) have increasingly replaced legacy polybrominated diphenyl ethers (PBDEs) in consumer products; however, concerns regarding their endocrine-disrupting properties continue to emerge. While introduced as safer alternatives, these replacements often exhibit significant trophic magnification and ubiquitous environmental detection. However, mechanistic data describing how emerging OPFRs (BPP, DPP, TBP, TP, and 2DPP) interact with the androgen receptor (AR) remain scarce. This study provides novel insights into the endocrine-disrupting effects of emerging OPFRs by addressing the limited knowledge regarding their activity toward the AR. Among these compounds, 2DPP, a component associated with commercial flame-retardant formulations, has been detected in environmental matrices and may pose risks to hormone-regulated biological processes. Despite increasing environmental occurrence, its effects on AR signaling remain poorly understood. We utilized an *in vitro* framework cascading from metabolic viability in MDA-kb2 cell lines. Functional AR-mediated luciferase reporter gene assay was done to assess the androgenic and anti-androgenic activities followed by mode of AR inhibition by performing shift assay. This methodology facilitates the identification of specific binding mechanisms, distinguishing allosteric interactions. Results identified distinct mechanism among the OPFRs. TP exhibited competitive antagonism, characterized by a parallel rightward EC₅₀ shift. On the other hand, BPP, DPP, TBP, and 2DPP demonstrated antagonism via allosteric sites, suppressing maximal response regardless of ligand concentration. 2DPP was particularly potent, inducing a >50% reduction in maximal fold induction. This allosteric silencing suggests that increasing endogenous androgens cannot overcome the endocrine blockade, a complex pathway often overlooked by traditional safety tests that fail to account for non-monotonic dose-responses. These findings provide crucial evidence required to prevent regrettable substitutions and support robust regulatory oversight of emerging environmental contaminants.

Keywords: Organophosphate flame-retardants, Androgen receptor, Antagonism, Endocrine disruptor, Non-competitive binding

Introduction:

The global phase-out of polybrominated diphenyl ethers (PBDEs) following their classification as persistent organic pollutants (POPs) has accelerated the adoption of organophosphate flame retardants (OPFRs) as alternative flame-retardant additives. However, growing evidence suggests that OPFRs may represent a regrettable substitution, as many exhibit environmental persistence, widespread human exposure, and potential endocrine-disrupting properties comparable to or exceeding those of legacy PBDEs (Blum et al., 2019). Unlike reactive flame retardants that are chemically bonded to polymer matrices, OPFRs are

primarily incorporated through physical blending, allowing their continuous release into the environment through volatilization, abrasion, and leaching from consumer products such as electronics, furniture, textiles, and food-contact materials (van der Veen and de Boer, 2012; Yang et al., 2019).

As a result, OPFRs have become ubiquitous environmental contaminants, frequently detected in indoor air, household dust, surface waters, sediments, wildlife, and human biological samples (Cequier et al., 2014; Chupeau et al., 2020). Recent monitoring studies have reported widespread occurrence of both legacy and emerging OPFR analogs, raising concerns regarding their bioaccumulation potential and long-term impacts on human health (Song et al., 2024). Of particular concern is their potential to disrupt endocrine signaling pathways, which regulate critical physiological processes including reproduction, development, and hormone homeostasis (Blum et al., 2019; Chupeau et al., 2020).

Despite increasing evidence of environmental exposure, the molecular mechanisms through which emerging OPFRs affect androgen signaling remain poorly understood. In particular, the interactions of BPP, DPP, TBP, TP, and 2DPP with the human androgen receptor (AR) have not been comprehensively characterized. Given the central role of AR signaling in male reproductive function, prostate physiology, and steroid hormone regulation, disruption of this pathway may have significant implications for human health. Therefore, this study employed a multi-tiered toxicological framework to investigate the endocrine-disrupting potential of emerging OPFRs and elucidate the molecular mechanisms underlying androgen receptor antagonism and steroidogenic interference.

Methodology:

This study employed an *in vitro* framework to comprehensively evaluate the endocrine-disrupting potential of emerging organophosphate flame retardants (OPFRs) using human-relevant cellular models. MDA-kb2 cells were used for androgen receptor (AR)-mediated transcriptional activity. Cell viability was assessed using the colorimetric MTT assay to ensure that the observed biological responses were due to endocrine disruption rather than nonspecific cytotoxic effects. AR transcriptional activity was quantified using a luciferase reporter gene assay in MDA-kb2 cells harboring the MMTV-neo reporter construct, enabling the identification of agonistic or antagonistic interactions with the receptor. In all assays, cells were treated with emerging OPFRs at concentrations ranging from 0.01 to 20 μ M. To evaluate potential androgenic effects, cells were treated with each test chemical for 24 hours without testosterone, whereas for anti-androgenic effects, cells were co-cultured with each emerging OPFR and 625 ($\sim$ EC₅₀) testosterone (P. Tachachartvanich et al., 2022).

To further characterize the mechanism of AR inhibition, a testosterone concentration-response shift assay was performed. MDA-kb2 cells were co-cultured to varying concentrations of testosterone alongside fixed concentrations of individual OPFRs, allowing differentiation between compounds that directly compete for the androgen receptor ligand-binding domain and those that exert effects through alternative or allosteric mechanisms. This comprehensive methodological approach minimizes confounding effects related to general cellular toxicity while offering a strong foundation for assessing receptor-mediated endocrine disruption.

Results and Discussion:

Cytotoxicity screening demonstrated that all tested organophosphate flame retardants (OPFRs) remained largely non-cytotoxic within the concentration range of 0.01–20 μM , providing a suitable experimental window for evaluating androgen receptor (AR)-mediated effects independent of general cellular toxicity. Luciferase reporter gene assays revealed that all five emerging OPFRs acted as AR antagonists, significantly suppressing testosterone-induced receptor activation in a dose-dependent manner. Among the compounds tested, 2DPP exhibited the greatest potency, reducing AR-mediated transcriptional activity to approximately 15–20% of the testosterone control at 20 μM while maintaining cellular viability. Notably, none of the compounds displayed agonistic activity, indicating an absence of receptor activation in the absence of endogenous androgen stimulation.

Mechanistic evaluation further distinguished the modes of AR inhibition exhibited by these contaminants. TP demonstrated characteristics of competitive antagonism, producing a parallel rightward shift in the testosterone dose-response curve consistent with direct competition at the receptor ligand-binding domain. In contrast, 2DPP, DPP, TBP, and BPP exhibited non-competitive or allosteric antagonistic behavior, suppressing maximal receptor activation without substantially altering EC_{50} values. These findings suggest that multiple emerging OPFRs interfere with AR signaling through alternative binding sites or mechanisms that reduce receptor functionality, highlighting a previously underappreciated pathway of endocrine disruption.

Unlike traditional competitive antagonists, which produce a parallel rightward shift in the dose-response curve, 2DPP decrease the maximal response. These findings indicate that 2DPP acts as a non-competitive antagonist potentially through allosteric site of the AR or interference with receptor associated signaling pathways.

Collectively, these results support the classification of emerging OPFRs as endocrine-disrupting chemicals capable of affecting AR signaling, emphasizing the need for mechanistic approaches in human health risk assessment.

Conclusions:

This study demonstrates that the transition from legacy polybrominated diphenyl ethers (PBDEs) to emerging organophosphate flame retardants (OPFRs) has introduced compounds with significant endocrine-disrupting potential. All investigated OPFRs acted as AR antagonists at non-cytotoxic concentrations, with 2DPP and DPP exhibiting particularly strong inhibitory activity. Distinct mechanisms of action were identified, including competitive antagonism by TP and non-competitive/allosteric inhibition by 2DPP, DPP, TB, and BPP, indicating that multiple pathways contribute to androgen signaling disruption. These findings provide mechanistic evidence that emerging OPFRs can interfere with receptor-mediated signaling, underscoring the limitations of conventional toxicity assessments that rely solely on apical endpoints. Consequently, the integration of mechanistic endocrine-disruption data into environmental risk assessment frameworks is essential for improving regulatory strategies and protecting human reproductive health.

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Environment / health impact assessment and management

A FIELD SURVEY AND ASSESSMENT OF ILLUMINATION AND LIGHT UNIFORMITY IN SUBDISTRICT HEALTH PROMOTING HOSPITALS OF NONTHABURI PROVINCE

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Abstract:

Despite being a frontline component of Thailand's public health system, subdistrict health promoting hospitals remain understudied with respect to indoor lighting quality and its implications for occupational health. This cross-sectional study described healthcare personnel characteristics and assessed indoor lighting quality in 30 subdistrict health promoting hospitals in Nonthaburi province, Thailand. Illuminance and lighting uniformity were measured in seven functional room types: examination room, procedure room, dental room, Thai traditional medicine room, office, meeting room, and waiting area. Additionally, self-reported health impacts related to illumination were collected from 115 healthcare personnel using a structured questionnaire. The results revealed that most clinical rooms failed to meet national illuminance standards. This was particularly critical in the procedure rooms (96.7%) and examination rooms (83.3%), spaces requiring visual precision. In contrast, the Thai traditional medicine rooms showed the highest compliance (95.0%), followed by the waiting areas (73.3%). Regarding light uniformity, distribution was consistently uniform across the majority of space categories, with the exception of waiting areas, meeting rooms, and offices. Notably, more than one-third of personnel (37.4%) reported light-sensitive conditions, predominantly among those working in the procedure rooms, examination rooms, dental rooms and offices. These findings provide critical evidence for occupational health policies aimed at improving illuminance adequacy and lighting distribution in primary healthcare facilities.

Keywords: Lighting quality, illuminance, uniformity ratio, health effect, hospital

Introduction:

Indoor lighting is a critical environmental factor affecting visual performance, psychological well-being, and occupational productivity. Beyond supporting visual tasks, lighting also plays an important role in regulating the human circadian system, influencing sleep quality, alertness, mood, and cognitive performance (CIE, 2019). As a result, healthy lighting has become a key component of building design, as reflected in international frameworks such as the WELL Building Standard and Human-Centric Lighting (HCL), which emphasize the importance of creating lighting environments that support both visual and non-visual human needs (IWBI, 2020). For healthcare personnel, appropriate lighting is particularly important because their work often requires prolonged concentration, visual precision, and direct patient care. Inadequate lighting can contribute to visual discomfort, fatigue, reduced work efficiency, and an increased risk of errors, potentially affecting the quality and safety of healthcare services. Therefore, lighting quality is not only a building design concern but also an important occupational and public health issue. Despite increasing global focus on healthy lighting environments, research in primary healthcare facilities remains limited, particularly in low- and middle-income contexts such as Thailand's subdistrict health promoting hospitals. Given that Nonthaburi Province's rapidly expanding urban and peri-urban populations require healthcare staff to manage high patient volumes and heavy workloads, it was chosen as the study setting to evaluate indoor lighting quality and describe its health impacts on personnel within these local facilities. Ultimately, addressing indoor lighting conditions is crucial for designing healthier and more efficient healthcare environments.

Methodology:

Study Design and Setting

This descriptive cross-sectional study was conducted across all 30 subdistricts health promoting hospitals affiliated with the Nonthaburi Provincial Health Office in Thailand. The research was granted ethical approval (COA No. MUPH 2026-030) from the Institutional Review Board of the Faculty of Public Health, Mahidol University.

Participants

A census approach was used, including all 136 health and public health personnel from the 30 subdistrict health promoting hospitals. Eligibility criteria were: (1) employed at the current facility for at least one year, (2) aged 20–60 years, (3) able to read and understand Thai, and (4) provided written informed consent. Personnel unavailable during the data collection period were excluded. A total of 115 participants met the eligibility criteria and provided complete data for analysis (response rate: 84.9%). Participants represented various clinical, public health, and administrative professions.

Illuminance Measurement

Indoor lighting quality was assessed in seven functional areas: examination room, procedure room, dental room, Thai traditional medicine room, office, meeting room, and waiting area. Illuminance was measured using a calibrated lux meter (Extech 407026) with a 2×2 m grid sampling method, in accordance with the Department of Labor Protection and Welfare notification. Lighting uniformity was evaluated using the uniformity ratio ($U_0 = \text{minimum illuminance/average illuminance}$) and compared against the recommended criterion of $U_0 \geq 0.60$ (EIT, 2021). Demographic and work-related information, as well as health effects on personnel, were collected via a structured questionnaire. Descriptive statistics were used to summarize participant characteristics and lighting conditions.

Results, Discussion and Conclusion:

Participant Characteristics (n = 115)

The majority of participants were female (87.8%), aged 25–34 years (40.0%), and worked as public health officers (41.7%), nurses (23.5%), or dental staff (12.2%). Most worked 7–9 hours/day (86.1%), five to seven days/week (95.7%), and had been in their current workspace for more than 12 years (82.6%). Over one-third of the staff (37.4%) reported light-sensitive conditions, including migraine (15.7%), dry eye (11.3%), and photophobia (11.3%). These conditions were **predominantly** found among specific groups, with **proportions** within each occupation reaching 48.1% for nurses, 41.7% for public health officers, and 28.6% for dental staff.

Lighting Quality Survey

Illuminance and uniformity findings are summarized in Table 1. Overall illuminance ranged from 45.8 to 1,021.5 lux. Most room types failed to meet national standards. The procedure rooms had the highest non-compliance rate (96.7%; mean 378.8 ± 221.3 lux), followed by the examination rooms (83.3%; mean 314.9 ± 155.9 lux) and dental rooms (77.3%; mean 375.2 ± 175.8 lux). In non-clinical areas, approximately 80% of offices and 50% of meeting rooms also failed to meet the ≥ 300 lux standard. Among these, the Thai traditional medicine rooms were the only area to consistently meet the standard, achieving 95.0% compliance, followed by the waiting areas at 73.3% compliance. **Regarding light uniformity, clinical zones generally achieved an acceptable level, with over 80% meeting the recommended guideline.** Conversely, the waiting areas showed the lowest compliance (34.5%), reflecting uneven light distribution. The meeting rooms displayed the widest variation in lighting uniformity (range 0.1–1.0), highlighting considerable heterogeneity in lighting conditions across facilities.

Table 1 Illuminance levels and uniformity ratios across 30 subdistrict health promoting hospitals, Nonthaburi Province.

Room Type	Illumination (Lux)			Uniformity Ratio			Standard (Lux)
	Max	Min	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD	
Examination Room	655.3	104.3	314.9 $\pm$ 155.9	1.0	0.4	0.8 $\pm$ 0.2	$\geq 500^*$
Procedure Room	1,021.5	85.8	378.8 $\pm$ 221.3	1.0	0.2	0.8 $\pm$ 0.2	$\geq 1,000^{**}$
Dental Room	832.3	98.3	375.2 $\pm$ 175.8	1.0	0.3	0.8 $\pm$ 0.2	$\geq 500^*$
Thai Med Room	813.8	45.8	240.5 $\pm$ 182.7	0.9	0.5	0.7 $\pm$ 0.2	$\geq 50^{***}$
Office Room	809.2	111.3	286.2 $\pm$ 179.0	1.0	0.3	0.7 $\pm$ 0.2	$\geq 300^*$
Meeting Room	701.0	82.7	292.9 $\pm$ 132.0	1.0	0.1	0.6 $\pm$ 0.2	$\geq 300^*$
Waiting Area	809.2	56.8	310.1 $\pm$ 195.3	0.9	0.3	0.6 $\pm$ 0.3	$\geq 200^{**}$

Note. Reference criteria: * International Commission on Illumination (CIE) EN 12464-1:2019; ** Thai Illuminating Engineering Association Guideline (TIEA-GD 003:2003); *** Thai Ministry of Labor, Ministerial Regulation on Safety, Occupational Health, and Work Environment Regarding Heat, Light, and Sound.

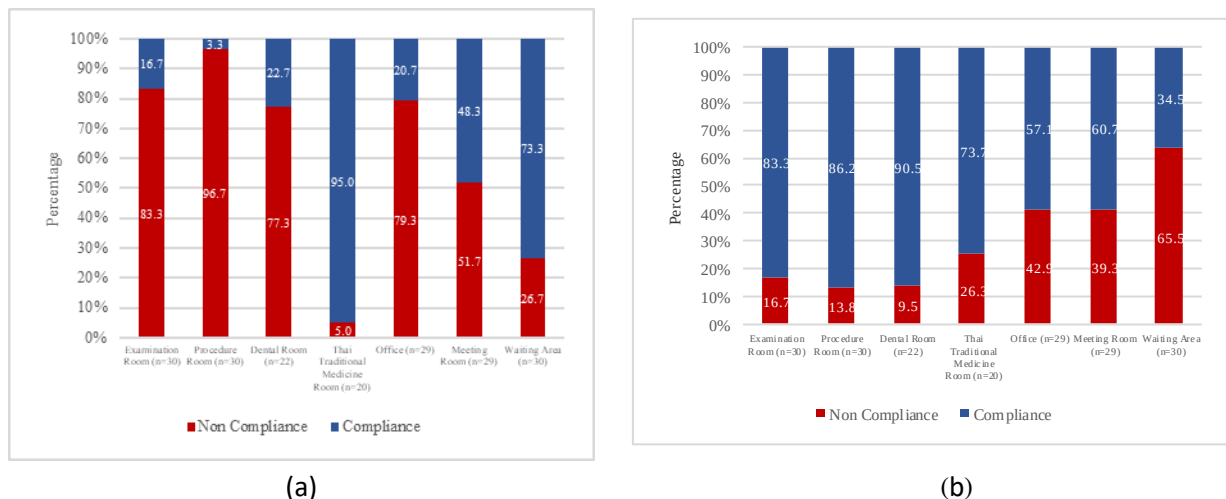


Figure 1 Lighting compliance status by room function (a) Illuminance and (b) Light Uniformity

Discussion:

Indoor illumination in most subdistrict health promoting hospitals in Nonthaburi Province, despite having good light distribution, did not comply with national standards. This was particularly evident in clinical areas requiring high visual precision—such as procedure, examination, and dental rooms—which aligned with reported symptoms of migraine, dry eye, and photophobia among the staff. Such conditions may also contribute to visual fatigue, reduced task accuracy, and potential risks to patient safety during clinical procedures (Figueiro et al., 2018). Furthermore, previous evidence showed that inadequate workplace lighting may disproportionately affect individuals with pre-existing visual or neurological sensitivities (WHO, 2018). Consequently, installing additional LED luminaires was recommended to raise overall illuminance to meet room-specific national standards. This would be supplemented by task-specific spotlights in areas requiring meticulous visual inspection, such as examination and procedure zones. In spaces with natural light, optimizing blinds and curtain adjustments, particularly in offices and meeting rooms, could serve as a cost-effective complementary measure to improve both illuminance adequacy and visual comfort.

Conclusion:

Although most clinical areas in Nonthaburi's subdistrict health promoting hospitals exhibited well-distributed light, evidence revealed inadequate illumination and negative health impacts. These findings provide evidence to support occupational health policies and healthy-building guidelines for primary healthcare facilities.

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FACTORS ASSOCIATED WITH INFECTIOUS WASTE MANAGEMENT BEHAVIORS AMONG HOSPITAL PERSONNEL: A CROSS-SECTIONAL STUDY USING THE PRECEDE–PROCEED MODEL

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Abstract:

Infectious waste management is essential for preventing healthcare-associated infections, protecting healthcare personnel and patients, and minimizing environmental contamination. Despite established regulations and guidelines, personnel practices remain a key determinant of effective infectious waste management. This study investigated the status of infectious waste management, assessed factors associated with waste management behaviors, and examined the relationship between these factors and infectious waste management behaviors among personnel at Khok Samrong Hospital, Lopburi Province, Thailand, using the PRECEDE–PROCEED Model as the conceptual framework.

A cross-sectional study was conducted among 140 hospital personnel selected by simple random sampling and 19 cleaning staff recruited through purposive sampling. Data were collected using a structured questionnaire, an infectious waste management quality assessment form, a hospital infectious waste management survey form, and a daily contamination record form. Descriptive statistics, the Chi-square test, and Fisher's exact test were used for data analysis, with statistical significance set at $p < 0.05$.

The hospital's infectious waste management system complied with the Ministerial Regulation on the Disposal of Infectious Waste B.E. 2545 (2002). Good knowledge of infectious waste management was observed among 59.3% of hospital personnel, while 97.8% demonstrated positive attitudes. Good infectious waste segregation practices were reported by 83.6% of hospital personnel, and 84.2% of cleaning staff demonstrated good collection and transportation practices. Personal factors, knowledge, and attitudes were not significantly associated with waste management behaviors ($p > 0.05$). However, previous education or training was significantly associated with appropriate infectious waste segregation behavior ($p = 0.002$).

These findings suggest that continuous capacity building through regular training, together with organizational supervision, monitoring, and institutional support, can effectively promote appropriate infectious waste management behaviors among hospital personnel and sustainably improve the efficiency of hospital infectious waste management systems.

Keywords: Infectious waste management, Hospital personnel, PRECEDE–PROCEED Model

Introduction:

Infectious waste is generated from medical and public health service activities and may be contaminated with pathogenic microorganisms, posing potential risks to healthcare

personnel, patients, and the environment. Improper management during waste segregation, collection, transportation, storage, and disposal may result in the transmission of infectious diseases and environmental contamination. Therefore, proper infectious waste management in accordance with scientific principles and legal regulations is essential for all healthcare facilities.

Hospitals are major sources of infectious waste. In 2025, Thailand generated more than 219,000 tons of infectious waste, while Lopburi Province generated 716.18 tons from 20 healthcare facilities, highlighting the need to improve infectious waste management systems in compliance with the standards of the Ministry of Public Health.

Khok Samrong Hospital, a 120-bed M2-level community hospital in Lopburi Province, generated 32.44 tons of infectious waste in 2025. Although the hospital has implemented a standardized infectious waste management system, problems such as improper waste segregation, mixing of infectious waste with general waste, and contamination of the wastewater treatment system were still identified, increasing the risk to healthcare personnel and the environment.

This study applied the *PRECEDE–PROCEED Model* (Green & Kreuter, 2005) to investigate the associations between predisposing, enabling, and reinforcing factors and infectious waste management behaviors among personnel at Khok Samrong Hospital, Lopburi Province. The findings are expected to provide evidence for improving infectious waste management practices and promoting sustainable healthcare waste management.

Methodology:

This study employed a cross-sectional study design to investigate the factors associated with infectious waste management behaviors among personnel at Khok Samrong Hospital, Lopburi Province, using the PRECEDE–PROCEED Model as the conceptual framework.

The study population consisted of 217 personnel involved in infectious waste management. A total of 140 personnel were selected using simple random sampling, while 19 cleaning staff directly responsible for infectious waste collection and transportation were recruited through purposive sampling. Research instruments included a structured questionnaire, an infectious waste management quality assessment form, a hospital infectious waste management survey form, and a wastewater treatment system contamination record form. All instruments were validated for content validity and reliability before data collection. Data were collected following approval from the Human Research Ethics Committee and analyzed using descriptive statistics, the Chi-square test, and Fisher's Exact test, with statistical significance set at $p < 0.05$.

Results, Discussion and Conclusion

Results

The findings revealed that Khok Samrong Hospital had implemented an infectious waste management system in compliance with the Ministerial Regulation on the Disposal of Infectious Waste B.E. 2545 (2002), covering the processes of waste segregation, collection, and transportation. The hospital generated an average of 68.1 kilograms of infectious waste per day, equivalent to approximately 2,104 kilograms per month. Nevertheless, the survey identified contamination of the hospital wastewater treatment system with floating debris and waste materials, including cloth scraps, wet tissues, plastic materials, and syringe caps or

needles. These findings indicate that improper waste segregation at the point of generation still occurred in some hospital departments.

Most participants demonstrated a good level of knowledge regarding infectious waste management (59.3%) and a good level of attitude toward infectious waste management (97.8%). Regarding enabling factors, the hospital provided adequate waste containers, equipment, and operational guidelines for infectious waste management. With respect to reinforcing factors, most personnel had previously received education or training related to infectious waste management; however, a proportion of personnel had never attended such training. Concerning waste management behaviors, 83.6% of hospital personnel demonstrated good infectious waste segregation practices, while 84.2% of cleaning staff demonstrated good practices in the collection and transportation of infectious waste.

The analysis of associations revealed that **personal characteristics**, including sex, age, educational level, marital status, job position, and duration of employment, as well as knowledge and attitudes regarding infectious waste management, were not significantly associated with infectious waste segregation behaviors ($p > 0.05$). In contrast, previous education or training related to infectious waste management was **significantly associated** with infectious waste segregation behaviors ($p = 0.002$). Furthermore, personal characteristics, knowledge level, attitudes toward infectious waste management, receipt of education, rewards, and disciplinary measures were not significantly associated with infectious waste segregation behaviors ($p > 0.05$).

Discussion:

The findings indicate that although Khok Samrong Hospital had established a standardized infectious waste management system and most personnel demonstrated good levels of knowledge, positive attitudes, and appropriate waste management behaviors, problems related to improper waste segregation and contamination of the wastewater treatment system were still observed. These findings suggest that the existence of a management system or standard operating procedures alone may not be sufficient to ensure appropriate practices without continuous supervision, monitoring, and regular educational reinforcement.

The absence of statistically significant associations between personal characteristics, knowledge, attitudes, and infectious waste management behaviors may be explained by the relatively homogeneous levels of knowledge and attitudes among hospital personnel, who performed their duties under the same institutional policies and operational standards. Consequently, little variation in waste management behaviors was observed. These findings are consistent with the PRECEDE–PROCEED Model (Green & Kreuter, 2005), which proposes that health-related behaviors are influenced not only by predisposing factors but also require support from enabling and reinforcing factors.

Conversely, previous education or training related to infectious waste management was significantly associated with appropriate waste management behaviors, indicating that continuous capacity building through regular training enhances personnel's understanding and promotes correct waste management practices. This finding is consistent with the study conducted by Athinyaporn Nithipanyakul (2020), which reported that education, training, and organizational support were important factors influencing infectious waste segregation behaviors among hospital personnel.

Conclusion:

Khok Samrong Hospital has implemented an infectious waste management system that complies with established national standards, and most personnel demonstrated good infectious waste management behaviors. Nevertheless, improper waste segregation and

contamination of the hospital wastewater treatment system were still identified, highlighting the need for continuous supervision, monitoring, and improvement of operational practices.

The findings demonstrated that education or training related to infectious waste management was an important factor significantly associated with personnel's waste management behaviors. Therefore, hospitals should provide regular education and refresher training programs, together with continuous supervision, performance evaluation, and improvements in equipment and workplace environmental support systems. These measures will enhance the efficiency of infectious waste management, reduce health risks to healthcare personnel, patients, and the environment, and contribute to the sustainable development of hospital infectious waste management systems.

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FACTORS INFLUENCING HOUSEHOLD WASTE SEPARATION BEHAVIORS AMONG MAE SOT MUNICIPAL RESIDENTS IN TAK PROVINCE, THAILAND

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Abstract:

This study aimed to identify the factors influencing household waste separation behavior in Mae Sot Municipality, Tak Province, Thailand, by applying the Theory of Planned Behavior (TPB). A structured questionnaire was administered to a randomly selected sample of 350 households across three communities within the municipality. Data were analyzed using descriptive statistics (mean, standard deviation) and regression analysis. Results indicated that residents demonstrated highly positive attitudes (mean 3.80) and strong intentions (mean 3.65) toward waste separation; however, their actual separation practices were only at a moderate level (mean 3.19). Regression analysis revealed that information availability, positive attitudes, and economic incentives explained 57.8% of the variance in intention. Crucially, the study identified a significant "intention-behavior gap," where high intention did not directly translate into actual behavior. Instead, the primary drivers for actual practice were facilitating conditions (such as the presence of categorized bins) and information availability, accounting for 62.6% of behavioral variance. These findings suggest that waste separation in Mae Sot is "facility-driven" rather than "motivation-driven," highlighting the need for improved municipal infrastructure and the restoration of public trust in the waste collection system.

Keywords: Household waste separation, Theory of Planned Behavior (TPB), Eco-industrial town, Facilitating conditions, Thailand

Introduction:

The management of municipal solid waste (MSW) in Thailand has become a critical environmental concern due to rapid urbanization. By 2023, waste generation was estimated at up to 95,728 tons per day, with organic waste comprising over 57% of the total. Improper disposal leads to severe public health threats, environmental pollution, and the emission of greenhouse gases. Despite government initiatives, household participation in source separation remains limited and inconsistent. Achieving integrated MSW management requires understanding people's norms, attitudes, and behavioral intentions, as well as providing adequate solid waste management (SWM) infrastructure from local government.

Mae Sot Municipality, Tak Province, is currently promoted as an eco-industrial town with a focus on sustainable waste-to-energy recovery. However, achieving these goals requires active community involvement in household-level waste separation. This study applied the Theory of Planned Behavior (TPB), focusing on attitudes, subjective norms, and perceived behavioral control, and expanded the framework to include economic incentives, information availability, facilitating conditions, and distrust of the waste collection system.

The research aims to explore current behaviors, explain related factors, and analyze the specific predictors that drive residents to separate waste in this unique border economic zone. While the TPB provides a framework focusing on attitudes and social norms, there is a lack of evidence regarding how factors like economic incentives, system distrust, and facilitating conditions interact to drive behavior in Mae Sot. Therefore, this study aims to analyze these specific predictors to provide evidence-based insights that can inform local policy and support the development of effective, context-sensitive waste management strategies. The objectives of this study are: i) to explore current waste separation behaviors, ii) to explain factors related to these behaviors, and iii) to analyze the determinants that influence the practice of waste separation.

Methodology:

This research employed a cross-sectional survey design. The target population consisted of household representatives in Mae Sot Municipality, totaling 41,590 households. The sample size was determined using Daniel's formula, resulting in 350 households. A stratified random sampling method was used to select three representative villages: Sak Thong (80 samples), Intarakhiree 1 (112 samples), and Donchai (158 samples). The research instrument was a three-part questionnaire: (1) General demographic information, covering gender, age, education, and income; (2) Psychological factors and intention, assessing attitudes, subjective norms, perceived control, and economic incentives using a 5-point Likert scale; and (3) Behavioral and external factors, measuring information availability, facilitating conditions, and actual separation practices. Content validity was confirmed by three experts with an Index of Item-Objective Congruence (IOC) ≥ 0.6 , and reliability was established with Cronbach's alpha ≥ 0.7 . Data were analyzed using Stata 14.1, employing descriptive statistics (mean, S.D.) and regression analysis to test nine hypotheses.

Results:

The analysis of individual factor influences on weights affecting waste separation intention is presented through the coefficients in Table 1. The results indicate that several predictors significantly contribute to the model, starting with a constant value of .861 ($p < .001$). Among the independent variables, the availability of information, categorized here within the distrust and intention metrics, shows the strongest positive influence with a standardized coefficient beta of .422 ($t = 8.516$, $p < .001$). This is followed by the intention factor itself, which carries a standardized beta of .382 ($t = 6.522$, $p < .001$). Perceived behavioral control also demonstrates a significant positive influence on waste separation intention with a standardized beta of .183 ($t = 3.393$, $p < .001$). Conversely, the factor relating to economic incentives shows a significant negative influence with a standardized beta of -.107 ($t = -2.955$, $p = .003$).

In contrast, certain factors did not show a statistically significant influence in this specific model, including attitude, which had a standardized beta of -.102 ($p = .093$), and subjective norm, which had a standardized beta of .020 ($p = .728$). Regarding the reliability of the model, collinearity statistics indicate that all variables remain within acceptable limits, with variance inflation factor (VIF) values ranging from 1.058 to 2.975, which are well below the standard threshold of 10, suggesting that there is no significant multicollinearity between the predictors. The tolerance values also range from .336 to .945, further supporting the stability of the regression coefficients.

Table 1 Analysis of individual factor influence weights affecting waste separation intention.

Predictor	B	SE	β	t	p	Tolerance	VIF
Constant	0.861	0.200	—	4.298	< .001	—	—
Intention	0.461	0.071	0.382	6.522	< .001	0.359	2.784
Attitude	-0.118	0.070	-0.102	-1.682	.093	0.336	2.975
Subjective Norm	0.019	0.054	0.020	0.348	.728	0.379	2.638
Perceived Behavioral Control	0.188	0.055	0.183	3.393	< .001	0.422	2.369
Economic Incentives for Household Waste Separation	-0.120	0.040	-0.107	-2.955	.003	0.945	1.058
Distrust of the Waste Collection System	0.385	0.045	0.422	8.516	< .001	0.501	1.997

Discussion:

The findings highlight a significant "Intention-Behavior Gap." Residents are mentally willing to separate waste but are hindered by situational constraints and systemic distrust. The moderate level of distrust (2.88) stems from the belief that collection staff ultimately mix separated waste (2.57) and that municipal problem-solving is slow. The dominance of facilitating conditions (Beta = 0.336) suggests that behavior in Mae Sot is "facility-driven." The availability of categorized bins and sufficient staff are more critical than individual willpower. Unlike studies in other regions where social pressure (subjective norms) drives behavior, Mae Sot residents view waste separation as an individual responsibility or a service-dependent task.

Conclusion:

Residents in Mae Sot practice waste separation at a moderate level (mean 3.19), primarily focusing on hazardous items rather than consistent recycling of paper and plastic. The study identifies a significant "Intention-Behavior Gap," where residents are willing to separate waste but are hindered by a moderate level of distrust (2.88) in the collection system, believing that separated waste is ultimately remixed by staff (2.57). Because behavior is "facility-driven," the municipality must move beyond awareness campaigns and prioritize infrastructure by increasing the number of categorized bins and ensuring convenient placement. To bridge the intention-behavior gap, local authorities should publicly demonstrate that waste remains separated during transport to restore trust. Communication strategies should utilize community broadcast towers and online media (Line/Facebook) for specific, bite-sized instructions. Finally, economic motivations should be leveraged through programs like "Waste for Eggs" or community recycling banks to provide tangible incentives for household participation.

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FOOD SANITATION KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG SCHOOL-VICINITY FOOD VENDORS: A CASE STUDY IN MIN BURI DISTRICT, BANGKOK

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Abstract:

Food sanitation among street food vendors surrounding schools is an important public health concern, particularly for school-aged children who are considered a vulnerable population. This cross-sectional study aimed to assess knowledge, attitudes, and practices (KAP) regarding food sanitation among food vendors and to examine the relationships among knowledge, attitudes, and practices regarding food sanitation.

A total of 63 food vendors operating around schools in Min Buri District, Bangkok, were selected using simple random sampling. Data were collected using a structured questionnaire, observational checklist. Descriptive statistics and Spearman's rank correlation coefficient were used for data analysis.

The results revealed that 60.32% of food vendors had a high level of knowledge, while 93.65% demonstrated positive attitudes toward food sanitation. However, 73.02% showed inadequate food sanitation practices. Knowledge was positively associated with attitudes ($r_s = 0.404$, $p = 0.001$). However, no significant association was observed between knowledge and practices ($r_s = 0.066$, $p = 0.607$), attitudes and practices ($r_s = -0.017$, $p = 0.893$).

The findings indicate that although food vendors possessed adequate knowledge and positive attitudes, these factors did not necessarily translate into appropriate food sanitation practices. Therefore, food safety interventions should focus not only on knowledge improvement but also on behavioral modification, practical training, and continuous monitoring programs.

Keywords: Knowledge Attitudes and Practices (KAP), Food Sanitation, Food Vendors, School-vicinity

Introduction:

Food sanitation is an essential component of food safety and environmental health. Inadequate food sanitation practices can increase the risk of foodborne diseases, particularly among children who are more vulnerable to contaminated food and poor hygiene conditions. The World Health Organization has emphasized that foodborne diseases remain a major global public health problem and are associated with unsafe food handling and poor sanitation practices (World Health Organization, 2015a, 2015^b).

Food stalls around schools provide affordable and accessible foods for students, teachers, and nearby communities. However, these food vending settings may face limitations related to space, equipment, water supply, waste management, and hygiene facilities. As a result, the food sanitation practices of vendors are important for reducing health risks in school food environments.

Min Buri District was selected as a study area because it has a large number of schools and a significant number of street food vendors surrounding it, making it a major food source for schools where students frequently purchase ready-to-eat meals. Min Buri District has some food hygiene monitoring, but it is not conducted consistently, resulting in limited data regarding the knowledge, attitudes, and practices of food vendors.

The knowledge, attitudes, and practices (KAP) framework has been widely used to assess food safety behaviors among food handlers. Previous studies have reported that food vendors may have moderate to high levels of food safety knowledge and positive attitudes, but actual food hygiene practices are not always consistent with their knowledge (Abid et al., 2022; Rahman et al., 2012). Therefore, this study assessed KAP regarding food sanitation among food vendors around schools in Min Buri District, Bangkok, and examined the relationships among knowledge, attitudes, and practices.

Methodology:

A cross-sectional study was conducted among food vendors operating around schools in Min Buri District, Bangkok. The study population consisted of 75 food vendors, and the sample size of 63 vendors was determined based on the Krejcie and Morgan (1970) sample size calculation for a finite population. Simple random sampling was used to select participants.

Data were collected using a structured questionnaire and an observational checklist. The questionnaire consisted of four parts: demographic characteristics, food sanitation knowledge, attitudes toward food sanitation, and food sanitation practices. Knowledge was assessed using 13 items, attitudes were assessed using 13 items on a five-point Likert scale, and practices were assessed using 20 items based on food sanitation criteria for food vending settings. The questionnaire was assessed for content validity by three experts using the Item-Objective Congruence (IOC) technique. The IOC values were 1.00 for the knowledge questionnaire and 0.76 for the attitude questionnaire. Reliability was evaluated through a pilot study involving 30 food vendors. The attitude questionnaire demonstrated excellent internal consistency (Cronbach's alpha = 0.98), while the knowledge questionnaire showed acceptable reliability using the Kuder-Richardson Formula 20 (KR-20 = 0.78). The study protocol was approved by the Human Research Ethics Committee of Mahidol University prior to data collection.

Descriptive statistics, including frequency, percentage, mean, and standard deviation, were used to describe the characteristics of participants and KAP levels. Spearman's rank correlation coefficient was used to examine the relationships between knowledge and attitudes, knowledge and practices, and attitudes and practices. Statistical significance was set at $p < 0.05$.

Results, Discussion and Conclusion:

A total of 63 food vendors participated in this study. Most participants were female (82.54%), aged over 40 years (71.43%), and had been operating food vending businesses for more than three years (85.71%). Most vendors sold ready-to-eat cooked foods (66.67%), and approximately half of the food stalls were located in front of schools (50.79%).

The overall mean knowledge score was 10.71 ± 1.95 out of 13. Most vendors had a high level of food sanitation knowledge (60.32%), followed by a moderate level (31.75%) and a low level (7.94%). Vendors had high knowledge regarding food covering, prevention of contamination from animals, and contamination risks from money handling. However, knowledge regarding the avoidance of jewelry and accessories during food handling was relatively low.

The mean attitude score was 4.28 ± 0.41 out of 5. Most vendors had positive attitudes toward food sanitation (93.65%). High attitude scores were observed for avoiding smoking during food preparation and covering the mouth and nose when coughing or sneezing. These results indicate that vendors generally recognized the importance of food sanitation and personal hygiene.

In contrast, the mean practice score was 10.32 ± 1.56 out of 20, and most vendors had practices that required improvement (73.02%). Only 26.98% of vendors had fair practices, while none achieved good or very good practice levels. This finding suggests a gap between knowledge, attitudes, and actual practices. Despite having good knowledge and positive attitudes, appropriate food sanitation practices may have been limited by inadequate personal hygiene, improper food handling, and the constraints of street food vending environments. These findings are consistent with previous studies reporting that adequate food safety knowledge alone does not necessarily translate into safe food handling practices, particularly when vendors face environmental and operational constraints (Abid et al., 2022; Rahman et al., 2012).

Table 1 Mean scores of knowledge, attitudes, and practices regarding food sanitation among food vendors.

Factor	Mean	SD	Interpretation
Knowledge	10.71	1.95	High level
Attitudes	4.28	0.41	Positive attitudes
Practices	10.32	1.56	Practices required improvement

Correlation analysis showed that knowledge was positively associated with attitudes ($r_s = 0.404$, $p = 0.001$), indicating that vendors with higher knowledge tended to have more positive attitudes toward food sanitation. However, knowledge was not significantly associated with practices ($r_s = 0.066$, $p = 0.607$), and attitudes were not significantly associated with practices ($r_s = -0.017$, $p = 0.893$). These findings suggest that knowledge and attitudes alone may not be sufficient to improve actual food sanitation practices.

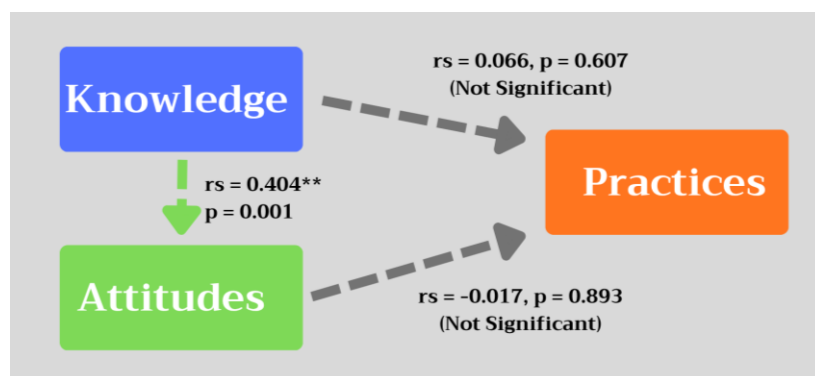


Figure 1 Relationships among Knowledge, Attitudes, and Practices Regarding Food Sanitation.

In conclusion, food vendors around schools in Min Buri District generally had good knowledge and positive attitudes regarding food sanitation, but their practices still required improvement. Practical training, continuous supervision, and regular monitoring should be implemented to strengthen food sanitation practices among food vendors around schools. These interventions may help improve the school food environment and reduce food safety risks among students.

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