



## Biofilms and antimicrobial-resistant foodborne bacteria in informal markets and street-vended ready-to-eat foods: Lessons and priorities for Cambodia

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**Abstract:** Foodborne infections remain a persistent public health challenge in low- and middle-income settings where informal markets and street-vended ready-to-eat foods play a major role in daily diets. In Cambodia, recent evidence from Phnom Penh city indicates substantial contamination of market foods and street-vended products with *Escherichia coli* (88% prevalence in ready-to-eat foods) and *Salmonella* (60% in street foods, 48.4% overall in market foods), suggesting significant exposure risks and gaps in food safety control. This narrative review synthesizes available evidence on microbial contamination in Cambodia's informal food sector, examines the role of biofilms in sustaining contamination on food-contact surfaces, explores the linkages between biofilms and antimicrobial resistance from a One Health perspective, and proposes practical, scalable surveillance and control options suitable for informal market settings. We conducted literature searches using PubMed, Web of Science, and Google Scholar for Cambodia-specific food safety studies and global literature on biofilm formation, persistence, and control in food environments. We included peer-reviewed publications, technical reports, and international guidance documents published between 2015 and 2025, with foundational earlier works. Available evidence from Phnom Penh (Cambodia) reveals high contamination prevalence in traditional markets and street-vended foods. The biofilm phenotype can enhance antimicrobial resistance through multiple mechanisms, including facilitation of horizontal gene transfer, sub-lethal antimicrobial exposure creating selection pressure, and harboring stress-tolerant cells. However, direct investigation of biofilm presence on food-contact surfaces in Cambodian markets has not been conducted, representing a critical research gap. Reducing foodborne disease risk and antimicrobial resistance transmission in Cambodia's informal food sector requires integrated actions addressing infrastructure, surveillance, and behavior change. Priority actions include expanding monitoring to include food-contact surface sampling; investing in water access and handwashing infrastructure; implementing vendor-focused training on proper cleaning sequences and equipment separation; piloting subsidized equipment upgrade programs; aligning with Codex Alimentarius frameworks for antimicrobial resistance surveillance; and conducting implementation research to identify feasible, effective interventions. Evidence is currently limited to Phnom Penh; geographic expansion and direct biofilm investigation represent important research priorities.

**Keywords:** Biofilm; Antimicrobial resistance; Street food; Informal markets; *Salmonella*; Cambodia; Food safety; One Health

### 1. Introduction

Antimicrobial resistance (AMR) represents one of the most urgent global health threats of the 21st century. Bacterial AMR was estimated to have directly caused 1.27 million deaths and contributed to 4.95 million deaths globally in 2019, with projections suggesting this burden will continue to escalate without coordinated intervention (Murray et al., 2022; World Health Organization, 2023). While AMR is most discussed in clinical healthcare settings, the food chain constitutes a critical yet often underappreciated pathway for the emergence, amplification, and transmission of resistant bacteria and resistance genes. Resistant organisms can move bidirectionally between animals, humans, and environmental reservoirs, making food safety an integral

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component of the One Health approach to combating AMR (Collignon et al., 2018; McEwen and Collignon, 2018).

Cambodia's food system is characterized by high reliance on traditional markets and street-vended foods, particularly in urban centers such as Phnom Penh. These informal food channels serve essential roles in providing affordable, accessible nutrition to large segments of the population and supporting the livelihoods of countless vendors and their families (Prak et al., 2018). However, recent integrative reviews of food safety in Cambodia have highlighted significant gaps in surveillance infrastructure, limited enforcement of food safety standards, and insufficient evidence to identify critical control points where interventions could most effectively reduce pathogen exposure (Thompson et al., 2021; Pok et al., 2020).

Within this context, biofilms deserve particular attention as a potential mechanism that may help explain persistent contamination patterns observed in informal food environments. Biofilms are structured microbial communities that attach to surfaces and become embedded in self-produced extracellular polymeric substances. These communities can allow foodborne pathogens to persist on food-preparation equipment, utensils, and surfaces, thereby repeatedly contaminating foods despite routine cleaning efforts (Myszka and Czaczyk, 2009; Chmielewski and Frank, 2003). The biofilm phenotype confers significant survival advantages, including enhanced resistance to physical removal, chemical disinfectants, and antimicrobial agents—properties that have profound implications for food safety control in resource-limited settings (Simões et al., 2010; Giaouris et al., 2014).

This narrative review focuses specifically on the intersection of biofilms, foodborne bacterial pathogens, and antimicrobial resistance within Cambodia's informal food sector. We synthesize available evidence on contamination patterns in traditional markets and street-vended ready-to-eat foods, examine the role of biofilms in sustaining contamination patterns, explore linkages between biofilms and AMR from a One Health perspective, and propose practical, evidence-based priority actions for surveillance and control that are feasible within the constraints of informal market settings.

## **2. Methods and Scope**

### **2.1. Review Approach**

This narrative review was conducted to integrate evidence from three complementary domains: Cambodia-specific empirical studies on microbial contamination of foods in traditional markets and street-vended products; global and regional scientific literature on biofilm formation, persistence, and control in food environments; and international guidance documents addressing antimicrobial resistance in the food chain. Unlike systematic reviews with exhaustive search strategies and meta-analysis, this narrative synthesis uses purposive sampling of literature guided by the authors' expertise to inform practical, priority actions for Cambodia's informal food sector.

### **2.2. Literature Search Strategy**

Literature research was conducted in December 2024 using PubMed, Web of Science, and Google Scholar databases. Search strategies combined geographic terms (Cambodia OR Cambodian), food safety terms (food safety OR foodborne disease OR street food OR informal markets), hazard terms (Salmonella OR *E. coli* OR contamination), and mechanism terms (biofilm OR antimicrobial resistance). Additional sources included direct review of the WHO and Codex Alimentarius websites, FAO technical reports, and forward/backward citation searching of key studies.

### **2.3. Inclusion Criteria and Selection**

We prioritized peer-reviewed publications from 2015–2025 while including seminal earlier works establishing foundational concepts in biofilm biology. Cambodia-specific empirical studies reporting contamination prevalence, surveillance data, or hygiene infrastructure assessments were included. Global literature on biofilm mechanisms, persistence, and control strategies was included when relevant to informal food settings. All screening and selection were conducted by the lead author, with verification by co-authors on ambiguous cases.



## 2.4. Evidence Synthesis

Evidence was organized using a farm-to-fork framework encompassing production, processing, distribution, retail, and household stages. Findings were synthesized narratively, emphasizing actionable insights relevant to public health laboratories, food safety regulators, and development partners working in low- and middle-income country contexts. The synthesis emphasizes feasibility and practical implementation over comprehensive academic coverage.

## 2.5. Limitations

This review has several important limitations. Cambodia-specific food safety evidence is limited to studies conducted primarily in Phnom Penh and surrounding areas; rural provinces and provincial cities remain understudied. No studies have directly investigated biofilm formation on food-contact surfaces in Cambodian markets; the connection between biofilm theory and Cambodia contamination patterns is inferred based on environmental conditions rather than empirically demonstrated. The narrative review methodology does not include systematic quality appraisal or quantitative synthesis. Generalizability of findings beyond Phnom Penh should be interpreted cautiously.

## 3. Cambodia Evidence: Contamination in Informal Markets

### 3.1. Traditional Market Contamination Patterns

Recent peer-reviewed evidence from Phnom Penh provides concerning data on *Salmonella* contamination across multiple food categories in traditional market settings. A comprehensive study sampling 285 food items—including meat, seafood, and leafy greens—from markets and farms reported an overall *Salmonella* prevalence of 48.4%, with significantly higher rates in animal-source foods: 71% in meat samples and 64% in seafood, compared with 33% in leafy green vegetables (Huoy et al., 2024). These findings indicate that contamination occurs at multiple points from primary production through transport, storage, and retail handling.

The study additionally documented substantial deficiencies in hygiene and sanitation infrastructure. Observed gaps included inadequate handwashing facilities, limited access to potable water, improper temperature control during storage and transport, and mixing of raw and ready-to-eat products without adequate separation or cleaning between handling different food types (Huoy et al., 2024). These conditions create environments conducive to initial contamination and to the establishment and persistence of microbial reservoirs on food-contact surfaces.

### 3.2. Street-Vended Ready-to-Eat Foods

Street-vended ready-to-eat (RTE) foods represent particularly high-risk exposure categories because they are consumed directly without subsequent heat treatment. A targeted study of 100 street-vended RTE food samples from Phnom Penh markets found *E. coli* in 88% of samples, indicating widespread fecal contamination, while *Salmonella* was isolated from 60% of samples (Chong et al., 2019). Papaya salad demonstrated particularly elevated *Salmonella* prevalence, likely reflecting the combination of raw vegetable ingredients, manual preparation involving extensive handling, use of shared cutting boards and knives, and lack of refrigeration during display and sale.

### 3.3. Environmental Conditions and Biofilm Risk

While these contamination patterns do not directly demonstrate biofilm presence, the environmental conditions documented—including compromised water quality, inadequate sanitation, insufficient surface cleaning, and repeated reuse of utensils without effective disinfection—are consistent with conditions favoring biofilm formation in other food service settings (Capita and Alonso-Calleja, 2013; Noor and Acharya, 2020). When cleaning is suboptimal, and surfaces remain moist with residual nutrients, bacteria can attach and begin forming a biofilm matrix within hours. Once established, biofilms become increasingly difficult to remove and can serve as persistent reservoirs continuously inoculating foods (Flemming et al., 2016). Direct investigation of biofilm presence on cutting boards, utensils, and preparation surfaces in Cambodian markets represents a critical research priority to validate this hypothesis.

## **4. Biofilms in Food Environments**

### **4.1. Biofilm Structure and Formation**

Biofilms are structured communities of microorganisms that adhere to surfaces and become encased in self-produced extracellular polymeric substances (EPS), typically comprising polysaccharides, proteins, extracellular DNA, and lipids (Costerton et al., 1999). In food environments, biofilms commonly form on cutting boards, knives, food processing equipment, drains, and countertops – particularly where moisture, nutrients, and surface characteristics support initial bacterial attachment and subsequent matrix development (Srey et al., 2013; Ban-Cucerzan et al., 2025).

Formation proceeds through stages: initial reversible attachment, transition to irreversible attachment, microcolony formation with EPS production, biofilm maturation with complex three-dimensional architecture, and dispersal of cells that can colonize new surfaces or contaminate foods (O'Toole et al., 2000). This process can occur rapidly, with attachment beginning within minutes to hours and mature biofilms developing within days.

### **4.2. Persistence Despite Cleaning**

Biofilms demonstrate remarkable resilience to physical and chemical removal. The extracellular matrix acts as a diffusion barrier, limiting penetration of cleaning agents and disinfectants. Cells within biofilms also exhibit altered metabolic states, with some entering dormant phases that inherently resist chemical antimicrobials (Bridier et al., 2011; Mah and O'Toole, 2001). Routine cleaning practices – particularly when abbreviated or performed without mechanical scrubbing – may remove surface contamination without eliminating underlying biofilm reservoirs, enabling continuous recontamination (Møretrø and Langsrud, 2017).

### **4.3. Enhanced Antimicrobial Tolerance**

Bacteria within biofilms can exhibit 10- to 1000-fold increased tolerance to antimicrobial agents compared with planktonic counterparts (Hall and Mah, 2017). This enhanced tolerance results from multiple mechanisms, including limited agent penetration through the EPS matrix, presence of persister cells with altered physiology, adaptive stress responses, and neutralization of antimicrobials by matrix components (Van Acker et al., 2014). For informal markets operating with limited access to hot water, effective detergents, and systematic cleaning protocols, these biofilm properties create situations where surfaces can harbor persistent contamination despite vendors' efforts to maintain cleanliness.

## **5. Biofilms and Antimicrobial Resistance**

### **5.1. Mechanisms Linking Biofilms to AMR**

The biofilm phenotype creates conditions that can facilitate both the emergence of antimicrobial resistance and the persistence and transmission of resistant strains. Mixed-species biofilm communities in food environments promote horizontal gene transfer through conjugation, transformation, and transduction, enabling resistance genes to spread between bacteria (Burmølle et al., 2006; Madsen et al., 2012). When cleaning agents or disinfectants fail to fully penetrate biofilms, bacteria in deeper layers experience sub-lethal concentrations that can select for resistant populations (Capita et al., 2014). Biofilms also harbor persister cell subpopulations that can survive antimicrobial challenges and repopulate surfaces after stress subsides (Pagedar et al., 2012; Lewis, 2010).

### **5.2. One Health Framework**

The One Health framework recognizes that human health, animal health, and environmental health are interconnected, requiring coordinated action across all domains (World Health Organization, 2023). Within this framework, the food chain represents a critical interface where resistant bacteria originating in food-producing animals can reach human populations, and where environmental contamination can enter food products (Food and Agriculture Organization, 2023). However, Cambodia-specific data integrating human, animal, and environmental sectors for AMR surveillance remains limited. Developing such integrated surveillance represents a priority for effective AMR control aligned with national action plans.

### **5.3. Codex Alimentarius Guidance**

Codex Alimentarius has developed frameworks for minimizing foodborne AMR, including the Code of Practice to Minimize and Contain Foodborne Antimicrobial Resistance (CXC 61-2005) and Guidelines on Integrated Monitoring and Surveillance of Foodborne AMR (CXG 94-2021) (Codex Alimentarius Commission, 2005, 2021).



These provide guidance on prudent antimicrobial use in food-producing animals and systematic collection and analysis of AMR data to inform risk management. For Cambodia, adopting Codex-aligned surveillance enables comparison with regional data, facilitates participation in international collaborations, and provides structured pathways for integrating food safety monitoring with national AMR action plans (FAO/WHO Codex Alimentarius, 2022).

## 6. Pathways from Informal Market Conditions to Biofilm Risk

Based on documented gaps in hygiene infrastructure observed in Cambodia's traditional markets and street food sector, several plausible pathways can be identified through which current conditions may promote biofilm formation:

Many informal market stalls operate with limited access to continuous running water, relying instead on stored water in buckets (Kov et al., 2020). This necessitates the reuse of washing water throughout the day, leading to progressive accumulation of organic matter and bacteria. Without sufficient water volume for thorough rinsing, residue remains on surfaces, providing nutrients that support bacterial attachment and biofilm development. When the water supply is intermittent, surfaces may remain damp for extended period conditions favoring biofilm maturation rather than elimination. Refrigeration infrastructure is often absent or inadequate in informal markets, resulting in extended storage at ambient tropical temperatures that support rapid bacterial multiplication (Roesel and Grace, 2014). When contaminated foods contact surfaces, high bacterial loads facilitate initial colonization. Residues left after food preparation provide both microbial inocula and nutrient substrates, enabling biofilm establishment. High throughput combined with limited equipment means the same cutting boards, knives, and utensils are frequently used sequentially for different food types—raw meats, seafood, vegetables, and ready-to-eat items—without effective cleaning between uses (Todd et al., 2008). This creates repeated cross-contamination opportunities, with each use depositing additional bacteria and organic material onto surfaces, progressively building biofilm communities. Traditional markets commonly employ wooden cutting boards and worn plastic surfaces with scratches, cuts, and cracks (Abrishami et al., 2020). These surface imperfections create protected microenvironments where bacteria become lodged and shielded from mechanical cleaning. Wooden surfaces are particularly difficult to clean effectively due to their porous nature and ability to retain moisture and nutrients. Once biofilms establish in these niches, simple wiping or rinsing becomes insufficient for removal. Poor drainage design can result in standing water, accumulated organic waste, and persistent moisture near food preparation areas (Carrascosa et al., 2021). Floor drains, if present, may harbor complex biofilm communities that contaminate preparation areas through splashing or aerosols. These environmental reservoirs can continuously reseed food-contact surfaces despite cleaning efforts.

## 7. Surveillance Approaches for Informal Markets

Effective surveillance in informal food settings requires approaches balancing scientific rigor with practical feasibility. A scalable surveillance package adaptable to public health laboratories could incorporate the following components:

### 7.2. Sampling Strategy

Priority should be given to ready-to-eat items consumed without additional heat treatment, such as papaya salad, fresh fruit preparations, and beverages. Critically, sampling should systematically include food-contact surfaces—cutting boards, knives, utensils, and preparation surfaces—to detect potential biofilm reservoirs. Paired sampling (both food and corresponding surfaces from the same vendors) enables investigation of whether contamination originates primarily from raw ingredients versus persistent surface reservoirs (Moore & Griffith, 2002). Water and ice used in beverages or food preparation should also be sampled.

### 7.3. Laboratory Testing

Indicator organisms (*E. coli*, total coliforms) serve as markers of fecal contamination and hygiene performance and are relatively inexpensive to enumerate (World Health Organization, 2022). Salmonella culture using selective enrichment followed by biochemical confirmation should be prioritized, given the documented high prevalence. When capacity allows, antimicrobial susceptibility testing should be performed on confirmed isolates using disk diffusion or other standardized approaches aligned with Clinical and Laboratory Standards



Institute guidelines (Clinical and Laboratory Standards Institute, 2023). For research purposes, basic biofilm characterization using microtiter plate assays can quantify biofilm-forming ability (O'Toole, 2011).

#### **7.4. Data Utilization**

Surveillance data have limited value unless translated into action. A risk-based approach would involve baseline assessment to identify the highest-risk foods, vendors, and locations; targeted intervention implementation; follow-up sampling to assess impact; and iterative refinement based on results. This implementation of a science approach generates evidence on effective, contextually appropriate control strategies.

### **8. Control and Prevention Strategies**

Biofilm control in industrial facilities relies on sophisticated systems unavailable in informal markets. Instead, a practical minimum package of interventions must address biofilm-related risks using simpler, accessible methods:

#### **8.2. Proper Cleaning Sequence**

The sequence of cleaning steps is critical for biofilm removal. Disinfectants applied to surfaces with residual debris have minimal effects. The correct sequence must begin with physical removal of gross contamination (scraping, rinsing) followed by detergent application with vigorous scrubbing using brushes to mechanically disrupt biofilm matrix (Gibson et al., 1999). Only after thorough rinsing should disinfectant be applied. Training materials should emphasize: scrape → wash with soap → scrub → rinse → sanitize → air dry. Approximate cost per vendor for basic cleaning supplies (soap, brushes): \$3-5 monthly.

#### **8.3. Contact Time and Concentration**

Disinfectants require adequate contact time at appropriate concentrations to be effective (Møretrø et al., 2017). Quick rinse practices typically fail to achieve sufficient contact time. Training should include simple practical methods, such as "count to 30" while sanitizer remains on surfaces. Disinfectant solutions must be prepared at correct concentrations using measuring tools and replaced regularly. Cost impact: proper dilution reduces waste and improves effectiveness without increasing expenditure.

#### **8.4. Equipment Replacement and Upgrades**

Heavily scarred wooden cutting boards and worn utensils should be replaced with non-porous, smooth materials that are easier to clean effectively (Welch et al., 2018). While upfront costs may seem prohibitive (plastic cutting boards: \$5-15 each; stainless steel knives: \$8-20), subsidized equipment upgrade programs or cooperative purchasing could make this feasible. Pilot programs in comparable Southeast Asian markets showed 60-70% vendor adoption when equipment subsidies were coupled with training. Even modest improvements in surface quality can substantially reduce biofilm attachment sites.

#### **8.5. Equipment Separation**

One of the most effective low-cost interventions is implementing strict separation between equipment used for raw foods and ready-to-eat foods (Food Standards Agency, 2023). This can be achieved through color-coding systems (red cutting boards for raw meat, green for vegetables, white for RTE foods) that are simple, visual, and do not require literacy. Cost per vendor for color-coded board set: \$15-25. Behavior change campaigns should emphasize that maintaining separation is among the most important food safety practices vendors can implement.

#### **8.6. Water Access and Handwashing Infrastructure**

Without reliable access to adequate clean water for handwashing, utensil cleaning, and food washing, even well-intentioned vendors cannot maintain adequate hygiene (Pickering et al., 2010). Infrastructure investments—installing handwashing stations with soap, ensuring continuous water supply, and providing appropriate drainage—are prerequisites for behavior change to be sustainable. Municipal-level investment required: approximately \$200-500 per handwashing station installation; \$50-100 monthly for soap and maintenance. Cambodia-focused reviews consistently emphasize that infrastructure and behavior change must proceed together (Pok et al., 2020; Kov et al., 2020).



### 8.7. Vendor-Centered Behavior Change

Behavior change interventions should be designed with vendor constraints in mind: short practical routines completable during busy periods; visual aids rather than text-heavy materials; positive messaging emphasizing vendor pride and customer trust; peer-to-peer learning and model vendor recognition programs; and regular supportive supervision rather than one-time workshops. Examples of simple behavioral targets include always washing hands before handling RTE foods, using separate boards for raw and cooked items, scrubbing cutting boards with soap and brush twice daily, and allowing utensils to air-dry rather than using potentially contaminated towels.

## 9. Research Priorities

To advance both scientific understanding and practical improvements in Cambodia's informal food sector, research priorities should emphasize feasible studies generating actionable evidence:

Future studies should systematically collect paired food and surface samples from the same vendors to investigate whether persistent surface contamination consistent with biofilm reservoirs explains food contamination patterns. Molecular typing methods could determine whether isolates from foods match those from surfaces, providing evidence for surface-to-food transmission. Biofilm formation assays on Cambodian isolates would quantify biofilm-forming capacity and identify high-risk strains (Erickson, 2012). Pragmatic intervention trials at the vendor or market level can evaluate whether feasible improvements result in measurable reductions in indicator organisms or pathogen prevalence. Study designs could employ before-and-after comparisons or matched controls, using microbial indicators as outcomes rather than requiring expensive clinical endpoint data (Arnold et al., 2013). These studies generate evidence on what works in real-world informal settings. Systematic antimicrobial susceptibility testing of foodborne isolates from markets and street foods, combined with epidemiological data on hygiene practices and infrastructure conditions, can identify associations between food safety gaps and AMR prevalence. This aligns with Codex guidance on integrated AMR surveillance (Codex Alimentarius Commission, 2021). Current evidence is limited to Phnom Penh. Investigations in rural provinces, provincial cities, and seasonal markets would enable an understanding of whether contamination patterns and control priorities differ by setting. Seasonal studies investigating how temperature, rainfall, and humidity influence contamination patterns could inform seasonally adapted messaging (Baker-Austin et al., 2018). Understanding economic feasibility from vendor and market authority perspectives is essential for achieving scale and sustainability. Research documenting costs of equipment upgrades, training programs, and infrastructure improvements, alongside potential benefits such as reduced food waste, enhanced customer trust, and avoidance of illness-related losses, can build the business case for investment by vendors, market associations, and municipal governments.

## 10. Conclusions

Cambodia's informal food sector, encompassing traditional markets and diverse street-vended ready-to-eat foods, provides essential nutrition and livelihoods but operates within constrained infrastructure and limited food safety oversight. Available evidence from Phnom Penh demonstrates substantial contamination of market foods and street-vended items with *Salmonella* and *E. coli* at prevalence levels indicating meaningful public health exposure risks. These contamination patterns signal not only acute foodborne illness risk but also potential pathways for antimicrobial-resistant organisms to reach human populations. Biofilms, structured microbial communities adhering to surfaces and protected by self-produced matrices, provide a plausible mechanistic explanation for persistent contamination despite routine cleaning efforts. The biofilm phenotype confers resistance to physical removal and chemical disinfection, enabling continuous recontamination from surface reservoirs. Moreover, biofilms can facilitate antimicrobial resistance emergence and transmission through multiple mechanisms, including horizontal gene transfer, sub-lethal antimicrobial exposure creating selection pressure, and harboring of stress-tolerant persisters. However, direct investigation of biofilm presence on food-contact surfaces in Cambodian markets has not been conducted, and current evidence is limited to Phnom Penh. Geographic expansion and empirical biofilm studies represent critical research priorities. Addressing biofilms and antimicrobial-resistant foodborne bacteria in informal markets requires moving beyond sporadic sampling studies toward sustained, integrated efforts combining infrastructure improvement, behavior change, surveillance, and policy development. The informal food sector is deeply embedded in

Cambodia's social and economic fabric and will continue playing a central role in feeding urban populations. Therefore, food safety improvements must be designed for these settings, not merely adapted from formal industry standards. Biofilms represent a potentially crucial but understudied factor in persistent contamination in low- and middle-income country food safety contexts. By investigating biofilm presence, incorporating surface monitoring, and implementing biofilm-appropriate control strategies, Cambodia can develop more effective approaches addressing root causes rather than symptoms. When coupled with attention to antimicrobial resistance and alignment with One Health and Codex frameworks, such efforts position Cambodia to make meaningful progress in protecting public health while supporting the livelihoods of those who depend on informal food systems.

### **Authors' Contribution**

Yem Sokha conceived the review topic, conducted the literature search, synthesized the evidence, and drafted the manuscript. Yim Sovannra contributed to literature screening and selection, data extraction, and drafting of sections. Kem Sokunthea contributed to critical appraisal, interpretation of evidence, and substantive revision. All authors read and approved the final manuscript.

### **Competing Interest**

The author declared no conflict of interest.

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### **AI Declaration**

Artificial intelligence tools (ChatGPT OpenAI) were used to support language editing, improve sentence clarity, and assist with structural refinement during manuscript revision. The authors reviewed and verified all content and take full responsibility for the accuracy, originality, and integrity of the work, including ensuring that all statements and citations are appropriate and scientifically sound.

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