Assessing factors of *E. coli* contamination of household drinking water in suburban and rural Laos and Thailand

N. Vannavong, H. J. Overgaard, T. Chareonviriyaphap, N. Dada, R. Rangsin, A. Sibounhom, T. A. Stenström and R. Seidu

**ABSTRACT**

Drinking water (DW) can serve as a route for disease transmission if not properly managed. The study assessed the effect of different factors on *Escherichia coli* quantities in DW in household water storage containers in suburban and rural villages in Laos and Thailand. Higher *E. coli* concentrations in DW were found in Laos compared to Thailand, especially in households without toilets (in Laos) and in rural rather than in suburban villages. In suburban Laos, house material, storage container types and lack of toilets were significantly associated with *E. coli* contamination of DW, whereas in rural Laos, corresponding significant factors were rain-fed water, containers with lids and lack of toilets. In suburban Thailand, rain-fed water, storage container types and container cleaning frequency were significantly associated with DW contamination, while house materials, manually collected rainwater and container cleaning frequency were associated with contamination in rural Thailand. Socio-demographic characteristics were less associated with *E. coli* contamination of DW in this study. Treatment of household stored water (e.g. boiling), regular cleaning of rain jars as well as the provision of household toilets, especially in Laos, can provide barriers against *E. coli* contamination of DW.

**Key words** | Escherichia coli, household drinking water, Laos, socio-demographic, Thailand

**INTRODUCTION**

Over the last decade, the diarrhoeal mortality among children under five has decreased globally from 1.2 million (in 2000) to 760,000 (in 2011), but 90% of these child deaths are still linked to water, sanitation and hygiene (UNICEF 2013). In Southeast Asia, 563,904 diarrhoeal deaths of all ages were estimated to be linked with inadequate water, sanitation and hygiene, which constitute 56% of diarrhoeal diseases in this region (Prüss-Ustün et al. 2014).

Storage of drinking water (DW) is a common practice in many countries where access to DW is either not available within the home environment or, if available, flows intermittently. DW can serve as a source of diarrhoeal disease transmission if not properly managed (WHO 2002). Among the causes of diarrhoeal disease incidence, the storage of DW within the household environment remains a significant risk factor (Roberts et al. 2001; Günther & Schipper 2013). All
Pathogens of viral, bacterial, parasitic and protozoan origins, implicated in gastroenteric disease outbreaks, may be found in the source water if contaminated, or introduced into the stored water due to poor handling.

In Laos and Thailand, many households have access to tap water, but prefer rainwater because the taste is considered better (Pinfold et al. 1993). Rainwater is collected during the wet season and stored throughout the year. Large cement jars (>2,000 L), introduced by governmental programs in the 1980s, are the main water storage containers in Thailand. Smaller jars of 200 L or less and plastic buckets are more common in Laos, but are also found in Thailand (Pinfold et al. 1995; Dada et al. 2013; Hiscox et al. 2015). A review on household DW in developing countries showed that stored water is often of a worse microbial quality than water from the source (Wright et al. 2004). Storage even for a short time can lead to microbial contamination of good-quality DW due to unhygienic handling. The stored DW in both Laos and Thailand was previously shown to partly be affected by microbial contamination with Escherichia coli (E. coli) (Dada et al. 2013) and did not comply with the World Health Organization (WHO) guideline of zero E. coli/100 mL (WHO 2006). A study conducted in Cambodia found a significant relationship between diarrhoeal disease and E. coli levels of ≥11 CFU/100 mL in DW compared with a reference E. coli level of <1 CFU/100 mL (Brown et al. 2008). Similar findings were made in rural households in South Africa and Zimbabwe where diarrhoeal disease was associated with E. coli levels of ≥10 CFU/100 mL in household DW collected from a communal source (Gundry et al. 2009). Other studies have found significant relationships between diarrhoeal disease incidence and the presence of E. coli in household DW (Jensen et al. 2004; Levy et al. 2012).

Several factors affect the quality of stored DW in the household environment. These factors include water collection and storage (Jensen et al. 2002; Clasen & Andrew 2005); hygiene and handling practices (Gundry et al. 2006; Eshcol et al. 2009; Rufener et al. 2010); and contamination at the source (Taneja et al. 2011; Too et al. 2016). There are few studies in Southeast Asia, especially in Laos and Thailand, on the main drivers of microbial contamination of stored DW in the household environment. However, for the development of cost-effective interventions, it is critical that factors contributing to the microbial contamination of DW in the household environment are identified. The main aim of this study is to assess these factors and their contribution to the microbial contamination of stored DW in suburban and rural villages in Laos and Thailand.

**MATERIALS AND METHODS**

**Study areas**

This study was conducted from February to April 2011 in Manchakhiri district, Khon Kaen province in northeastern Thailand, and from May to June 2011 in Lakhonpheng district, Salavan province in southern Laos (Figure 1). Using existing health data and in consultation with each country’s public health departments, one suburban and one rural village per country were selected based on previously described criteria (Dada et al. 2013).

**Study design and data collection**

The study was a cross-sectional survey involving household interviews, observations and water sampling. A systematic random sampling procedure was used in the selection of study households from a total of 215 and 130 households in the suburban and rural villages in Laos, and from a total of 272 and 139 households in the suburban and rural villages in Thailand, respectively. This resulted in the inclusion of 121 and 114 households from suburban and rural Laos, and 117 and 121 households from suburban and rural Thailand, respectively. General characteristics of the households were obtained through interviews using semi-structured questionnaires administered to household heads. Information collected included socio-demographic characteristics (Tables 1 and 2). DW sources (e.g. rain-fed water (rainwater that is collected directly from the rooftop, through the roof-connected tube or from a metal roofing sheet), manually collected rainwater (rainwater that is collected manually from large containers), purchased bottled water, and borehole water), water management practices, sanitation facilities and hygiene. This was further supplemented by observations to ascertain the types of toilet facilities that were in use, presence of soap at handwashing
facility, types of water storage containers, presence or absence of a secured lid on the water storage containers and mode of collecting water to drink. The interviews and observational surveys were conducted together with trained field staff (village health volunteers). The DW samples were collected from a total of 139 and 145 water containers in

Figure 1 | Study villages in Laos and Thailand (Dada et al. 2013).
suburban and rural Laos. In Thailand, 178 and 268 DW samples were collected from the suburban and rural villages, respectively. Samples were collected using sterile 100 mL Whirl-Pak bags, put on ice and transported to a field laboratory where they were analyzed for E. coli within 24 h after sampling. E. coli analysis was done using Colisure-Quantitray/2000 method (Colisure® WCLS2001, IDEXX Laboratories, Inc., Westbrook, ME, USA). Details of the E. coli analysis and identification procedures are described in Dada et al. (2016). E. coli results were expressed as most probable number (MPN)/100 mL.

E. coli was chosen as a faecal indicator in this study because it is easy and less costly to analyze; and is identified as the most suitable indicator of faecal contamination in DW. Also, it is generally considered as a reliable indicator for the presence or absence of other pathogenic bacteria such as Salmonella, Shigella and Campylobacter spp. (WHO 2017). Although thermotolerant coliforms are also recommended and used as alternative group of faecal indicator organisms, a review showed that E. coli, rather than thermotolerant coliforms, in household DW was significantly associated with diarrhoea (Gruber et al. 2014).

Data analysis

Descriptive analysis was undertaken to examine the statistical distribution (frequency, percentage, central tendency and rate) of factors related to socio-demographic characteristics, DW, water management, sanitation and hygiene. The mean, minimum and maximum of E. coli (MPN/100 mL) concentration related to all study factors were displayed. Households were ranked into rich, intermediate and poor ones using principal components analysis and based on group weighted mean scores (Vyas & Kumaranayake 2006). The variables used in the wealth status ranking are presented in Table 1. A univariate analysis was undertaken to assess the independent effect of these factors on the occurrence of E. coli in the household DW. Regardless of significance, all factors included in the univariate analysis were entered into a zero-inflated negative binomial (ZINB) multivariate model to assess the effect of multiple factors on the occurrence of E. coli in the DW in households. The ZINB model was used to account for over-dispersion and excess zeroes in E. coli values. From the multivariate ZINB analysis, incidence rate ratios (IRR) and their 95% confidence intervals were obtained. The IRRs of factors significantly affecting (p < 0.05) E. coli concentration in the DW from the univariate and multivariate analysis are presented. All statistical analyses were conducted using STATA (version 12; STATA Corporation, College Station, TX, USA).

RESULTS

Socio-demographic characteristics of households

The socio-demographic characteristics of the study households are presented in Table 2. Over 90% of the household members in both countries were literate and the main occupation was agriculture, especially in the rural villages in both countries (over 94%). Households in Thailand were wealthier than in Laos. In Thailand, most houses were made of cement–wood (70% in suburban and 61% in rural village); while in Laos, the houses were mainly made of wood alone (42% in suburban and 85% in rural village).

DW sources, management practices and levels of E. coli contamination

The main source of DW in both suburban Thailand and Laos was purchased bottled water (Combined Table 5a and 5b)
whereas rainwater collection was the most common both in rural Thailand (directly or drawn from the collection jars) and in rural Laos (manually drawn from the collection jars).

Generally, the highest levels of \(E.\ coli\) contamination in DW were recorded in the rural villages compared to the suburban villages (Table 3a and 3b). The levels of \(E.\ coli\) contamination was higher in Laos than in Thailand especially in rain-fed water (mean: 117.7 MPN/100 mL, min.: 0, max.: 1,986 MPN/100 mL) in suburban Laos and manually collected rainwater in rural Laos (98.4 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL) (Table 3a). The suburban village in Thailand had the lowest mean \(E.\ coli\) contamination level (3.7 MPN/100 mL, min.: 0, max.: 105 MPN/100 mL) compared to all other sites (Figure 2). Purchased bottled water and borehole (not available in Thailand and rural Laos) were the least contaminated across study villages except in suburban Laos, with a mean of 112.2 MPN/100 mL, min.: 0, and max.: 2,420 MPN/100 mL (Table 3a).

Although the majority of the containers in all villages had lids (80–98%) they were unprotected from contamination, especially in both rural villages. The concentration of \(E.\ coli\) was high in stored DW in households that collected water by scooping compared to those that poured

### Table 2 | Household characteristics in suburban and rural villages in Laos and Thailand

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<thead>
<tr>
<th></th>
<th>Laos</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
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<td>Suburban</td>
<td>Rural</td>
</tr>
<tr>
<td>No. of households</td>
<td>121</td>
<td>114</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>40 (33.1)</td>
<td>47 (41.2)</td>
</tr>
<tr>
<td>Female</td>
<td>81 (66.9)</td>
<td>67 (58.8)</td>
</tr>
<tr>
<td>Literate</td>
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<tr>
<td>Yes</td>
<td>110 (90.9)</td>
<td>104 (91.2)</td>
</tr>
<tr>
<td>No</td>
<td>11 (9.1)</td>
<td>10 (8.8)</td>
</tr>
<tr>
<td>Occupation</td>
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<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td>63 (52.1)</td>
<td>108 (94.7)</td>
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<tr>
<td>Commerce</td>
<td>12 (9.9)</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>Service</td>
<td>30 (24.8)</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>Other(^a)</td>
<td>16 (13.2)</td>
<td>0</td>
</tr>
<tr>
<td>Room occupancy rate</td>
<td></td>
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</tr>
<tr>
<td>(&lt;2.5) persons/room</td>
<td>61 (50.4)</td>
<td>71 (62.3)</td>
</tr>
<tr>
<td>(&gt;2.5) persons/room</td>
<td>60 (49.6)</td>
<td>43 (37.7)</td>
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<td>Wealth status</td>
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<tr>
<td>Poor</td>
<td>39 (32.2)</td>
<td>92 (80.7)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>49 (40.5)</td>
<td>16 (14.0)</td>
</tr>
<tr>
<td>Rich</td>
<td>35 (27.3)</td>
<td>6 (5.3)</td>
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<tr>
<td>Housing material</td>
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<td></td>
</tr>
<tr>
<td>Cement and wood</td>
<td>45 (37.2)</td>
<td>16 (14.0)</td>
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<tr>
<td>Cement</td>
<td>25 (20.7)</td>
<td>1 (0.9)</td>
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<tr>
<td>Wood</td>
<td>51 (42.1)</td>
<td>97 (85.1)</td>
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<tr>
<td>Cement and wood</td>
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<td>14 (12.3)</td>
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<tr>
<td>Cement</td>
<td>37 (30.6)</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Wood</td>
<td>53 (43.8)</td>
<td>94 (82.5)</td>
</tr>
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<td>Ground</td>
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<td>0</td>
</tr>
<tr>
<td>Mode of collecting water to drink</td>
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<td></td>
</tr>
<tr>
<td>Pouring into cup</td>
<td>79 (65.3)</td>
<td>75 (65.8)</td>
</tr>
<tr>
<td>Scooping into cup</td>
<td>42 (34.7)</td>
<td>39 (34.2)</td>
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<tr>
<td>Sanitation facility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pour flush toilet</td>
<td>77 (63.6)</td>
<td>25 (21.9)</td>
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<tr>
<td>Flush toilet</td>
<td>7 (5.8)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>No toilet</td>
<td>37 (30.6)</td>
<td>88 (77.2)</td>
</tr>
<tr>
<td>Hand washing facility with soap near or inside the toilet</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>80 (66.1)</td>
<td>26 (22.8)</td>
</tr>
<tr>
<td>No</td>
<td>41 (33.9)</td>
<td>88 (77.2)</td>
</tr>
</tbody>
</table>

Percentages in parentheses.
\(^a\)Retired, unemployed and student.
Table 3 | Contamination of *E. coli* in stored DW (*E. coli* MPN/100 mL) related to socio-demographic, DW sources, household water management, sanitation and hygiene in suburban and rural villages in Laos and Thailand

<table>
<thead>
<tr>
<th>Suburban</th>
<th>Rural</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>Mean (min–max)</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
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<tr>
<td>No. of containers</td>
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**Socio-demography**

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<tr>
<th>Study locations</th>
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<tr>
<td>Literacy</td>
<td>124</td>
<td>130</td>
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<tr>
<td>No</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

**Occupation**

| Agriculture | 77  | 139 | 76.6 (0–2,420) |
| Commerce    | 12  | 3   | 2.3 (0–4)       |
| Service     | 33  | 3   | 0.7 (0–2)       |
| Othera      | 17  | 0   |                 |

<table>
<thead>
<tr>
<th>Room occupancy rate</th>
<th>72</th>
<th>93</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2.5 persons/room</td>
<td>36.6 (0–1,986)</td>
<td>104.6 (0–2,420)</td>
</tr>
<tr>
<td>&gt;2.5 persons/room</td>
<td>83.5 (0–2,420)</td>
<td>17.9 (0.222)</td>
</tr>
</tbody>
</table>

**Wealth status**

| Poor        | 49  | 120 | 85.8 (0–2,420) |
| Intermediate| 57  | 19  | 15.7 (0–179)   |
| Rich        | 33  | 6   | 11.5 (0–53)    |

| Cement and wood | 52  | 19  | 19.4 (0–179) |
| Cement         | 26  | 1   | 0             |
| Wood           | 61  | 125 | 82.5 (0–2,420) |

| Rain-fed      | 19  | 34  | 18.9 (0–222) |
| Manually collected rain | 22  | 101 | 98.4 (0–2,420) |
| Purchased bottled water | 70  | 10  | 7.6 (0–53) |
| Borehole      | 25  | 0   |               |

**Housing material**

| Jar           | 26  | 46  | 27.6 (0–548) |
| Bottle        | 33  | 44  | 67.8 (0–2,420) |
| Bottle        | 66  | 30  | 33.4 (0–579) |
| Jug           | 8   | 15  | 179.9 (0–2,420) |
| Others        | 3   | 6   | 450.8 (0–2,420) |
| Without lid   | 4   | 29  | 11.2 (0–107) |
| With lid      | 135 | 116 | 89.1 (0–2,420) |

| Pouring into cup | 84  | 98  | 64.6 (0–2,420) |
| Scooping into cup | 55  | 47  | 92.2 (0–2,420) |

| At least biweekly | 137 | 142 | 75.1 (0–2,420) |
| Monthly          | 0   | 2   | 0.5 (0–1)      |
| Monthly to yearly | 2   | 1   | 1.0 (1–1)      |

| Pour flush toilet | 86  | 28  | 6.4 (0–55)    |
| Flush toilet     | 7   | 1   | 3.0 (3–3)     |
| No toilet        | 46  | 116 | 90.3 (0–2,420) |

**Sanitation facility**

| Hand washing facility with soap near or inside the toilet | No  | 52  | 70.1 (0–1,986) | 117 | 88.5 (0–2,420) |
| Yes           | 87  | 28  | 10.9 (0–179)   |

(continued)
Table 3 | continued

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<th>Suburban</th>
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b. Thailand

<table>
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<tr>
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Socio-demography

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</tr>
<tr>
<td>Commerce</td>
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<td>2.5</td>
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<tr>
<td>Service</td>
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<td>1.7</td>
</tr>
<tr>
<td>Othera</td>
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<th>Room occupancy rate</th>
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<td>&gt;2.5 persons/room</td>
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<thead>
<tr>
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<tr>
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<td>88.4</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5.5</td>
<td>35.8</td>
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<tr>
<td>Rich</td>
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<thead>
<tr>
<th>Housing material</th>
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<tr>
<td>Cement and wood</td>
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<tr>
<td>Cement</td>
<td>4.8</td>
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<tr>
<td>Wood</td>
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<th>Sources of DW (most used)</th>
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<tr>
<td>Rain-fed</td>
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<td>Manually collected rain</td>
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<td>Bottled water</td>
<td>3.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Borehole</td>
<td>5.0</td>
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Household water management

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<tr>
<th>Types of DW storage containers (most used)</th>
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<tbody>
<tr>
<td>Jar</td>
<td>3.2</td>
<td>25.9</td>
</tr>
<tr>
<td>Bucket</td>
<td>6.4</td>
<td>61.8</td>
</tr>
<tr>
<td>Bottle</td>
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<td>1.0</td>
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<tr>
<td>Jug</td>
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<td>1.1</td>
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<td>With lid</td>
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<td>264</td>
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<table>
<thead>
<tr>
<th>Mode of collecting water to drink</th>
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</thead>
<tbody>
<tr>
<td>Pouring into cup</td>
<td>2.9</td>
<td>47</td>
</tr>
<tr>
<td>Scooping into cup</td>
<td>4.1</td>
<td>31.2</td>
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<tr>
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<td>At least biweekly</td>
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<tr>
<td>Monthly</td>
<td>1.9</td>
<td>189.6</td>
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<tr>
<td>&gt;Monthly to yearly</td>
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<tr>
<th>Sanitation facility</th>
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<th>265</th>
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<tbody>
<tr>
<td>Pour flush toilet</td>
<td>3.4</td>
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<td>Flush toilet</td>
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<td>Hand washing facility with soap near or inside the toilet No</td>
<td>2.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Yes</td>
<td>3.9</td>
<td>36.2</td>
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</tbody>
</table>

Min-max: minimum–maximum.

*aRetired, unemployed and student.
from containers to drink, except in rural Thailand (Table 3b). Nearly all containers in Laos (98%) were reported to be cleaned at least biweekly. In Thailand, however, containers were cleaned less frequently; 50% of suburban containers and 50% of rural containers were cleaned monthly or less frequently (Table 3b).

**Types of DW containers**

Bottles were the most commonly used DW containers in suburban Laos, followed by buckets, while in the rural village jars and buckets were used equally (Table 3a). In Thailand, generally, jars and buckets were the most commonly used DW containers, with jars being the most predominant in the rural village (Table 3b). Jugs and containers grouped as ‘other’ were more common in Laos than in Thailand, but were the least used across all sites (Table 3a and 3b). Of the most common containers, jars in suburban Laos had the highest mean contamination levels (145.7 MPN/100 mL, min.: 0, max.: 1,986 MPN/100 mL), followed by buckets in rural Laos (67.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL), and buckets in rural Thailand (61.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL). However those less commonly used, i.e. jugs and ‘other’ had the highest mean E. coli contamination level than all of the containers inspected, 179.9 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL and 450.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL, respectively (Table 3a).

**Sanitation and hygiene**

In Laos, 77.2% of the households in rural villages had no toilet facilities compared with 30.6% in suburban villages. In Thailand, nearly all households (100% in suburban and 98.3% in rural village) had toilet facilities (Table 2). The proportions of households with toilets and handwashing facilities with soap in suburban and rural Laos were 66.1% and 22.8%, respectively. In suburban and rural Thailand, the proportion of households with toilets, in addition to handwashing facilities, was 83.8% and 98.3%, respectively.

Households without toilets had higher levels of E. coli contamination in their stored DW than households with...
toilets (Table 3a). In Laos, the mean E. coli concentrations in DW in suburban and rural villages without toilets were 145.8 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL) and 90.3 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL), respectively. In Thailand, nearly all households had toilets and the average levels of E. coli in stored DW was generally lower than in Laos (Table 3b). In addition, households without hand washing facility with soap near or inside the toilet in Laos had higher E. coli concentrations in DW than those with soap. In suburban Laos, the mean E. coli concentrations in DW for households without hand washing facility with soap and those households with handwashing facilities was 70.1 MPN/100 mL (min.: 0, max.: 1,986 MPN/100 mL) and 52.7 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL). In rural Laos, the mean concentration of E. coli in drinking water was 88.5 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL) for households without handwashing facilities and 10.9 MPN/100 mL (min.: 0, max.: 179 MPN/100 mL) for households with handwashing facilities (Table 3a).

Univariate analysis

Table 4 presents the significant factors associated with the occurrence of E. coli in stored DW from the univariate analysis. In suburban Thailand, none of the factors were significantly associated with the occurrence of E. coli in DW. The occupation of household heads was significantly associated with the occurrence of E. coli in DW in both rural Laos and Thailand (Table 4). Generally, DW in poor wealth status households was more likely to be contaminated with E. coli than DW in higher wealth status households (intermediate and rich). In rural Laos, DW in the intermediate wealthy households was less likely to be contaminated with E. coli than DW in the poor households (IRR: 0.2; CI: [0.0-0.9]; p = 0.045). Similarly, DW in rich households were less likely to be contaminated with E. coli than DW from poor households in suburban Laos (IRR: 0.01; CI: [0.0-0.1]; p < 0.001) and rural Thailand (IRR: 0.1; CI: [0.0-0.4]; p = 0.001). In rural Laos, households with more than 2.5 persons per habitable room were less likely to have E. coli contaminated DW compared to those with less than 2.5 persons per habitable room (IRR: 0.1; CI: [0.0-0.4]; p < 0.001). In rural Thailand, E. coli contamination of stored DW was associated with the type of housing material. In this regard, DW stored in houses made of cement were more likely to be contaminated with E. coli than DW stored in houses made from both wood and cement (IRR: 10.5; CI: [2.7-40.3]; p = 0.001). Similarly, DW stored in houses constructed with only wood were more likely to be contaminated with E. coli than water stored in houses made from both wood and cement (IRR: 9.0; CI: [3.5-23.3]; p < 0.001).

With respect to DW sources, some water sources in both rural villages were significantly associated with the occurrence of E. coli in DW. In rural Laos, rain-fed water and purchased bottled water were significantly less likely to be contaminated with E. coli (IRR: 0.2; CI: [0.0-0.6]; p = 0.005) and (IRR: 0.1; CI: [0.0-0.9]; p = 0.039), respectively. However, containers manually filled with rainwater were more likely to be contaminated with E. coli compared to those that were not (IRR: 8.9; CI: [2.9-26.7]; p < 0.001). Similarly in rural Thailand, containers manually filled with rainwater were also more likely to be contaminated with E. coli compared to those that were not (IRR: 11.9; CI: [5.5-25.9]; p < 0.001).

Among the household water management practices, the type of storage containers and mode of collecting water to drink were the only factors significantly associated with the occurrence of E. coli in DW, but only in Laos (Table 4). In suburban and rural Laos, buckets and jars, as storage containers, were less likely to be contaminated with E. coli than other kinds of containers. In suburban Laos, DW in households where members scooped water from containers was more likely to be contaminated with E. coli than those pouring the water out (IRR: 4.3; CI: [1.1-16.1]; p = 0.033). In rural Laos, DW in containers covered with lids were more likely to be contaminated with E. coli than those without lids (IRR: 9.2; CI: [2.4-35.7]; p = 0.001).

Water containers in households without a toilet facility were more likely to be contaminated with E. coli compared to households using pour flush toilet in Laos (Table 4). In suburban and rural Laos, households without a toilet facility were over four times (IRR: 4.8; CI: [1.3-18.1]; p = 0.02) and 14 times (IRR: 14.2; CI: [3.5-56.9]; p < 0.001) more likely to be contaminated with E. coli compared to households using pour flush toilets. In rural Laos, DW containers in households with a handwashing facility with soap near or inside the toilet were significantly less likely to be contaminated with E. coli (IRR: 0.2; CI: [0.0-0.6]; p = 0.008) compared to those without a handwashing facility with soap.
Table 4  | RR (95% confidence intervals) p-value by univariate analysis of E. coli in stored DW (E. coli MPN/100 mL) related to socio-demographic, DW sources, household water management, sanitation and hygiene in suburban and rural villages in Laos and Thailand

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<th>Laos Rural</th>
<th>Thailand Suburban</th>
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<tr>
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<td>0.7 [0.0–17.5] 0.815</td>
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<tr>
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<td>0.04 [0.0–2.4] 0.128</td>
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<tr>
<td>Other^</td>
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<td>0.02 [0.0–0.8] 0.035</td>
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<tr>
<td>&gt;2.5 persons/room 1.4 [0.4–5.7] 0.609</td>
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<td>0.8 [0.2–2.6] 0.696</td>
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<td>0.9 [0.0–58.3] 0.997</td>
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<td>Rich 0.01 [0.0–0.1] &lt; 0.001</td>
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<td>0.4 [0.1–1.0] 0.062</td>
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<td>9.0 [5.5–23.3] &lt; 0.001</td>
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<td>Yes 0.2 [0.0–1.2] 0.078</td>
<td>8.9 [2.9–26.7] &lt; 0.001</td>
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<td>11.9 [5.5–25.9] &lt; 0.001</td>
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<td>bottled water</td>
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<td>0.1 [0.0–0.9] 0.039</td>
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<td>Yes 2.2 [0.4–12.9] 0.369</td>
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<td>Types of DW storage containers</td>
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<td>Yes 3.7 [0.7–19.5] 0.123</td>
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<td>Bucket</td>
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<tr>
<td>Yes 0.1 [0.0–0.7] 0.014</td>
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<td>2.2 [0.9–5.8] 0.096</td>
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<td>Yes 1.1 [0.3–4.6] 0.869</td>
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<td>without lid</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>with lid na</td>
<td>9.2 [2.4–35.7] &lt; 0.001</td>
<td>na</td>
<td>14.5 [0.4–47.4] 0.133</td>
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<tr>
<td>Mode of collecting water to drink</td>
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<tr>
<td>Pouring into cup 4.3 [1.1–16.1] 0.035</td>
<td>1.6 [0.4–6.0] 0.453</td>
<td>0.7 [0.2–2.5] 0.607</td>
<td>0.7 [0.3–1.8] 0.47</td>
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<td>Scooping into cup</td>
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<td>Frequency of container</td>
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<td>At least biweekly 1</td>
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<td>1</td>
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<td>Monthly 0.01 [0.0–1.2] 0.058</td>
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<td>&gt;Monthly to yearly na</td>
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<td>1.5 [0.4–5.9] 0.517</td>
<td>1.0 [0.4–2.4] 0.994</td>
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(continued)
Multivariate analysis

As with the univariate analysis, results from the multivariate analysis revealed that the main factors contributing to the occurrence of *E. coli* in stored DW at the household level was country specific and varied from location to location (Table 5).

DW stored in wooden houses were more likely to be contaminated with *E. coli* in suburban Laos (IRR: 8.2; CI: [1.9–34.9]; *p* = 0.004) and rural Thailand (IRR: 2.7; CI [1.1–6.5]; *p* = 0.031), than DW stored in houses made from both cement and wood.

Rain-fed water in rural Laos and suburban Thailand, and manual filling of containers with rainwater in rural Thailand were significantly associated with the occurrence of *E. coli* in DW containers (Table 5). Specifically, in rural Thailand, the occurrence of *E. coli* in stored DW was nearly 34 times more likely in households manually filling their containers with rainwater than in households that were not (IRR: 33.6; CI: [9.2–122.5]; *p* < 0.001). DW in rain-fed containers were less likely to be contaminated with *E. coli* than manually filled containers in rural Laos (IRR: 0.2; CI: [0.1–0.9]; *p* = 0.044), but the opposite was found in suburban Thailand (IRR: 33.5; CI: [1.4–771.5]; *p* = 0.028).

DW containers with lids were nearly six times more likely to be contaminated with *E. coli* than those without lids in rural Laos (IRR: 5.6; CI: [1.3–24.5]; *p* = 0.023). There was a higher occurrence of *E. coli* contamination in DW stored in jars compared with other storage containers in suburban Laos (IRR: 8.9; CI: [0.9–79.4]; *p* = 0.05) and suburban Thailand (IRR: 23.0; CI: [2.2–244.4]; *p* = 0.009). Also, storage of DW in buckets increased the occurrence of *E. coli* contamination in suburban Thailand (IRR: 4.9; CI: [1.1–22.5]; *p* = 0.041), whereas in suburban Laos, water stored in bottles were more likely to be contaminated with *E. coli* compared with non-bottled water (IRR: 20.7; CI: [4.0–106.0]; *p* < 0.001). In suburban Thailand, DW containers that were cleaned less frequently (monthly and longer, up to yearly), were less likely to be contaminated with *E. coli* compared with those that were cleaned more frequently (IRR: 0.01; CI: [0.0–0.6]; *p* = 0.026). However, in rural Thailand, containers that were less frequently cleaned (monthly and longer, up to yearly) were more likely to be contaminated with *E. coli* than containers that were cleaned more frequently (IRR: 7.9; CI: [2.2–28.5]; *p* = 0.001). The occurrence of *E. coli* in stored DW was around seven times (IRR: 7.2; CI: [1.6–31.5]; *p* = 0.009) and nearly 17 times (IRR: 16.6; CI: [5.8–65.3]; *p* < 0.001) more likely in households without a toilet facility compared with households using pour flush toilet in suburban and rural Laos, respectively.

**DISCUSSION**

The occurrence of *E. coli* in DW storage containers at the household level in Laos and Thailand was a function of several factors that varied from village to village. These factors resulted in high concentrations of *E. coli* (>1,000 *E. coli* MPN/100 mL) in stored DW across the study areas in...
both countries. The exception to this was suburban Thailand, where the concentrations of E. coli in DW were lower (\(\leq 105\) E. coli MPN/100 mL). Stored DW in containers in Laos had higher E. coli contamination levels than in Thailand. The relatively high E. coli concentrations in the two villages in Laos and in rural Thailand are a potential risk factor for gastroenteric diseases, particularly diarrhoea. Children in the study areas, especially those less than 2 years could potentially be at risk of diarrhoeal disease transmission as observed in an earlier study conducted in the Philippines (Moe et al. 1994).

Socio-demographic characteristics such as occupation of household heads, room occupancy rate, and wealth status were significantly associated with the occurrence of E. coli in stored DW only in the univariate analysis. Furthermore, housing material was significantly associated with E. coli in the

Table 5 | RR (95% confidence intervals; p-value) by multivariate analysis of E. coli in stored DW (E. coli MPN/100 mL) related to socio-demographic, DW sources, household water management and sanitation in suburban and rural villages in Laos and Thailand

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<td>Wood</td>
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</tr>
<tr>
<td>Types of DW storage containers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jar</td>
<td>No</td>
<td>1</td>
<td>8.9 [0.9–79.4]</td>
<td>23 [2.2–244.4]</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.05</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Bucket</td>
<td>No</td>
<td>1</td>
<td>4.9 [1.1–22.5]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottle</td>
<td>No</td>
<td>1</td>
<td>20.7 [4.0–106.0]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lid status</td>
<td>Without lid</td>
<td>1</td>
<td>5.6 [1.3–24.5]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With lid</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of container cleaning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least biweekly</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Monthly</td>
<td>0.01 [0.0–0.5]</td>
<td>8.6 [0.6–121.7]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.026</td>
<td>0.112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;Monthly to yearly</td>
<td>7.9 [2.2–28.5]</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitation facility</td>
<td>Pour flush toilet</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Flush toilet</td>
<td>0.5 [0.0–4.9]</td>
<td>0.4 [0.0–249.5]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No toilet</td>
<td>7.2 [1.6–31.5]</td>
<td>16.6 [3.8–65.5]</td>
<td></td>
</tr>
</tbody>
</table>

IRRs in bold are significant at \(p \leq 0.05\).
multivariate analysis especially in suburban Laos and rural Thailand, where the mean \( E. coli \) concentrations in DW containers were higher if house construction material was wood (Table 3a and 3b). Wooden houses in all study villages were generally characterized by low wealth status. Low wealth status was significantly associated with an increased occurrence of \( E. coli \) in DW (Table 4) and, therefore, could have high occurrence of \( E. coli \) in their stored DW compared with houses constructed with both cement and wood (Table 5).

Rainwater, which is frequently used in all villages, was significantly associated with \( E. coli \) contamination in both the univariate and the multivariate analysis. DW containers with rain-fed water in rural Laos were significantly less likely to be contaminated with \( E. coli \) but this was not the same for suburban Thailand where rain-fed water had a significantly higher level of \( E. coli \) contamination. In the study areas in Laos, rain-fed water was directly collected through connected pipes from rooftops. As a result, the chance of household members dipping their hands into water during collection is much lower compared with containers manually filled by hand. Furthermore, only small rain jar containers (200 mL) that are cleaned frequently on a biweekly basis are used to store rainwater in rural Laos. Manually collected rainwater in rural Thailand also had a higher level of \( E. coli \) (Table 5). Rain-fed water in suburban Thailand and the manually collected rainwater in rural Thailand is unsafe to drink and therefore a considerable risk factor because of the increased occurrence of \( E. coli \) contamination as well as \( E. coli \) contamination levels not meeting the WHO standards (WHO 2006). In suburban Laos, although borehole water is less used compared to rainwater and purchased bottled water, it had the highest levels of \( E. coli \) contamination (Table 3a) and also a higher likelihood of contamination (IRR: 4.8; CI: [0.9–25.9]; \( p = 0.067 \)) in the multivariate analysis compared to non-borehole water (Table 5).

Previous studies have shown that household water management play a role in \( E. coli \) contamination of stored DW (Jensen et al. 2002; Clasen & Andrew 2003). This was also confirmed in our current study where household water management practices were associated with \( E. coli \) contamination of stored DW (Table 5). In rural Laos, although nearly 100% of DW containers were said to be cleaned at least biweekly and 80% of them were covered with lids (Table 3a), the containers covered with lid had a significantly more occurrence of \( E. coli \) contamination (Tables 4 and 5). This is in contrast with findings made in previous studies (Chida-vaenzi et al. 1998; Mazengia et al. 2002), where faecal and total coliform counts were 50% lower in containers covered with a lid compared to those without a lid. This may be explained by the fact that nearly 70% of DW in rural Laos came from the manually collected rainwater contaminated with high numbers of \( E. coli \) during the collection process (Table 3a). It has been shown that \( E. coli \) counts can increase with duration or time of storage (Jenkins et al. 2011). Also contamination of the water source could also explain this (Jensen et al. 2002; Wright et al. 2004). However, it should be stressed that as this was a cross-sectional study, the time-varying effect of \( E. coli \) levels in containers with and without lids was not accounted for. There is therefore a need for further studies accounting for the effect of water sources, as well as duration of stored water, on \( E. coli \) contamination in containers with and without lids.

In suburban and rural Thailand, the frequency of cleaning containers was significantly associated with the occurrence of \( E. coli \) in stored DW. In rural Thailand, less frequent cleaning of containers (once per month or less frequent) increased \( E. coli \) contamination of DW compared with biweekly cleaning (Table 5). The most commonly used DW containers in rural Thailand were rain jars (Table 3b), which are usually up to 2,000 L (Dada et al. 2013). Containers of this size or capacity are not easy to clean; hence, water storage in such containers could lead to deterioration in water quality over time. In contrast to rural Thailand, containers that were less frequently cleaned (cleared monthly and less often than monthly) in suburban Thailand were significantly less likely to be contaminated with \( E. coli \) (Table 5). A low room occupancy rate (Table 2) as well as access to other sources of DW such as bottled water in suburban Thailand might explain this (Table 3b). A low room occupancy rate combined with access to other sources of DW reduces household members’ interaction/contact with stored DW, thereby reducing the potential for contamination.

This study revealed that access to improved toilet facilities could provide a significant barrier against the contamination of stored DW within the household environment. Lack of access to toilet facilities was significantly associated with \( E. coli \) contamination of DW. This was particularly evident
in Laos where a significant proportion of households in suburban and rural villages were without toilet facilities (Table 2). DW in households without toilets in suburban and rural Laos had high levels of E. coli contamination (Table 3a), and were significantly associated with the occurrence of E. coli in DW in the univariate and the multivariate analysis. Members of households without toilet facilities often resort to open defaecation without any handwashing facilities, and are therefore more likely to contaminate stored DW. Also the improper containment of human excreta resulting from the lack of toilet facilities can potentially provide other pathways for the contamination of stored DW within the household environment; and increase the risk of diarrhoeal disease transmission (Tumwine et al. 2002).

CONCLUSIONS

We conclude that the concentration of E. coli in stored DW in the household environment is higher in Laos than in Thailand, especially in households without toilets in both villages in Laos, as well as in the rural rather than suburban villages of both countries. Different factors contribute to the deterioration of water quality, and these vary across villages. From the final analysis, the factors that were significantly associated with the occurrence of E. coli in DW in suburban Laos were wooden house material, jars and bottles, and households without toilets; whereas, in rural Laos, the factors were rain-fed water, containers covered with lids and households without toilets. In suburban Thailand, significant factors associated with the occurrence of E. coli in DW were rain-fed containers, jars, buckets and container cleaning frequency; whereas, in rural Thailand, they were wooden house material, manually collected rainwater and container cleaning frequency. This study revealed that socio-demographic characteristics were less associated with E. coli contamination in DW, compared to sanitation and hygiene. This may not always be the case in every setting, as all of these factors (Table 5) generally have been associated with E. coli, hence, each or any combination of these factors serve as potential risk factors for faecal contamination. The levels of E. coli contamination found in all study sites were above the WHO drinking-water quality guidelines. Health education for appropriate treatment of stored DW (e.g. boiling) prior to drinking can provide a significant barrier against diarrhoeal disease incidence in households with poor water quality. In Laos, interventions related to the provision of improved toilet facilities have the potential of improving the quality of stored DW.

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