Enteric etiological surveillance in acute diarrhea stool of United States Military Personnel on deployment in Thailand, 2013–2017

Woradee Lurchachaiwong1*, Oralak Serichantalergs1, Paphavee Lertsethtakarn1, Nattaya Ruamsap1, Apichai Srijan1, Wirote Oransathid1, Nuanpan Khemnu1, Brian A. Vesely1, Samandra T. Demons1, Norman C. Waters1, John M. Crawford1 and Brett E. Swierczewski2

Abstract

Background: Diarrhea remains a major public health problem for both civilian and military populations. This study describes the prevalence of acute diarrheal illness etiological agents, their antibiotic resistance distribution patterns, the resulting impact upon military force health protection, and potential prevention and treatment strategies.

Results: Forty-eight acute diarrhea stool samples from US military personnel deployed to Thailand from 2013–2017 were screened for enteric pathogens using ELISA, the TaqMan Array Card (TAC), and conventional microbiological methods. These isolates were also evaluated using antimicrobial susceptibility testing (AST) against ampicillin (AMP), azithromycin (AZM), ceftriaxone (CRO), ciprofloxacin (CIP), nalidixic acid (NA), erythromycin (ERY), and trimethoprim-sulfamethoxazole (SXT) using commercial methodology. Susceptibility results were interpreted following the CLSI and NARM guidelines. Questionnaire data obtained from 47/48 volunteers indicated that 89.4% (42/47) reported eating local food and the most common clinical symptoms were nausea and abdominal pain (51%; 24/47). Multiple bacterial species were identified from the 48 stool samples with diarrhea etiological agents being detected in 79% (38/48) of the samples distributed as follows: 43.8% (21/48) Campylobacter jejuni and Campylobacter species, 42% (20/48) diarrheagenic Escherichia coli, and 23% (11/48) Salmonella. Co-infections were detected in 46% (22/48) of the samples. All C. jejuni isolates were resistant to CIP and NA. One C. jejuni isolate exhibited resistance to both AZM and ERY. Lastly, an association between exposure to poultry and subsequent detection of the diarrhea-associated pathogens E. coli and P. shigelloides was significant (p < 0.05).

Conclusion: The detection of Campylobacter isolates with CIP, AZM and ERY resistance has critical force health protection and public health implications, as these data should guide effective Campylobacteriosis treatment options for deployed military members and travelers to Southeast Asia. Additional research efforts are recommended to determine the association of pathogen co-infections and/or other contributing factors towards diarrheal disease in military and traveler populations. Ongoing surveillance and AST profiling of potential disease-causing bacteria is required for effective disease prevention efforts and treatment strategies.
Keywords: Enteric etiology, Acute diarrhea, United States Military Personnel, Thailand

Background
Exposure to enteric pathogens is one of the major causes of diarrheal infections in both traveler and military populations [1]. Previous studies have reported that military personnel acquire infectious diarrhea during military exercises [2]. The risk of diarrheal infection is regionally dependent, particularly for civilian travelers and military personnel in transition from industrialized countries into developing countries [3, 4]. Reported incidence rates for bacterial diarrheal disease in military and travelers caused by enterotoxigenic Escherichia coli (ETEC), Campylobacter, and Shigella were between 38 and 45% in previous reports from various countries [5]. Typical treatment for traveler’s diarrhea includes the use of antibiotics to include ciprofloxacin, azithromycin and rifaximin [6]. However, enteric pathogens and their associated antibiotic resistance patterns evolve over time and vary by region [7, 8]; therefore, access to up-to-date data on the global epidemiology of present diarrheal agents and their respective resistances are vital for diminishing the risk of diarrheal infection [6].

There are five Pacific region countries with which the US has a functional security alliance, including Thailand. The Armed Forces Research Institute for Medical Sciences (AFRIMS), based in Thailand, has coordinated studies of deployed US military to Thailand (i.e., the annual US–Thai “Cobra Gold” joint military forces exercise) for several years. Documented studies from previous exercises in Thailand demonstrated that US soldiers suffer consistent diarrhea attack rates during their first few weeks in country [9–11]. Despite modern preventive methods, diarrhea remains a primary concern for force health protection and therefore mission success for deployed military personnel in Thailand. Thus, the main objective of this study was to report the prevalence and clinical symptoms of diarrheal etiologic agents and bacterial pathogen antimicrobial susceptibility (AST) patterns affecting deployed US military personnel in Thailand for Cobra Gold exercises conducted in 2013 to 2017. This information will be useful in formulating more effective prevention and treatment strategies for these acute illnesses in deployed US forces.

Methods
Study design
A prospective acute diarrhea study was conducted in February of each calendar year, 2013–2017, at the following field sites: Lopburi, Phitsanulok, Chonburi (Samae-sarn/Utapao), and Chanthaburi (Baan Chan Khem).

Diarrheal cases were defined as three or more loose stool in the previous 24 h, starting no more than 72 h before presentation, with concurrent clinical symptoms such as nausea, vomiting, and abdominal or bowel pain. After obtaining informed consent, US military service members who presented with these criteria and symptoms self-reported on an administered questionnaire the following: stool frequency and description, poultry exposure, local food consumption, and any additional clinical symptoms. Stool grading (formed, soft, loose, or watery) was assessed by US military medical staff. The stool grading “loose” is described the stools that appear softer than normal whereas “watery” is specified to the stools appearance that no solid pieces and all liquid. The study was approved yearly by the Walter Reed Army Institute of Research institutional review board, Silver Spring, Maryland, USA.

Laboratory methods
Microbiological methods
Approximately 4–10 grams of stool was collected from each subject. Stool samples were tested for the presence of enteric bacteria pathogens by inoculating onto the following media: MacConkey agar (MC), Heiktoen (HE), thiosulfate citrate bile salt sucrose (TCBS), modified semi-solid Rappaport–Vassiliadis (MSRV), modified charcoal ceferopazone deoxycholate agar (mCCDA), buffered peptone water, alkali peptone water and Preston selective enrichment broth. Cellulose acetate membrane (Sartorius, Germany) was used to filter inoculated stool samples on Brucella agar plate (BAP) with sheep blood. Subsequently, identification of Shigella, Salmonella, Vibrio, Aeromonas, Plesiomonas, Yersinia, Campylobacter and Escherichia coli was performed as previously described [12].

Antimicrobial susceptibility testing
Isolated enteric pathogens, except Campylobacter and Arcobacter, were evaluated by AST following standard Kirby–Bauer method to the following antibiotics, ampicillin (AMP), azithromycin (AZM), ceftriaxone (CRO), ciprofloxacin (CIP), nalidixic acid (NA) and co-trimoxazole (SXT), using commercially prepared discs according to the manufacturer’s instructions (Becton, Dickinson and Company, USA). Susceptibility results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Campylobacter and Arcobacter isolates were evaluated using E-tests (Biomérieux, NC, USA). The minimal inhibitory concentration (MIC) was...
defined as the lowest concentration of an antimicrobial agent that completely inhibited visible growth and was read at the point where the elliptical zone of inhibition intersected the MIC scale on the strip. Due to the limitation of CLSI guidelines for *Campylobacter*, the National Antimicrobial Resistance Monitoring System (NARM) 2013 criteria for AZM, CIP, erythromycin (ERY) and NA were followed for these isolates [14].

**Multiplex polymerase chain reaction (multiplex-PCR)**
Isolated 5–10 lactose fermenting colonies were inoculated onto MacConkey agar (MC) and Hektoen (HE) media, cultured for 18–24 h at 37 °C, and sub-cultured onto trypticase soy agar (TSA) with 5% sheep blood. Lactose-fermenting colonies were picked for nucleic acid by boiling extraction method. Identification of diarrheagenic *Escherichia coli* (Enteropathogenic *E. coli* (EPEC) [15], Enteroinvasive *E. coli* (EIEC) [16], Enteraggregative *E. coli* (EAEC) [17, 18], Enterotoxigenic *E. coli* (ETEC) [19–21], and Enterohemorrhagic *E. coli* (EHEC) [15, 22] was performed using multiplex PCR.

**Enzyme-linked immunosorbent assay (ELISA)**
Qualitative Enzyme-linked immunosorbent assay (ELISA) kits (TechLab, Inc., USA) were utilized to detect *Giardia lamblia*, *Cryptosporidium*, and *Entamoeba histolytica* in fecal specimens. ELISA kits (Ridascreen® and R-Biopharm; Germany) were used for the detection of rotavirus, astrovirus, adenovirus, and *Campylobacter* according to the manufacturer’s instructions.

**TaqMan® array card**
Total nucleic acid was extracted from frozen stool using the QiaAmp stool DNA kit (Qiagen, Valencia, California) and used in the Enteric Pathogen TaqMan® Array Card (TAC) as previously described [23]. Briefly, 40 μL of extracted nucleic acid from each stool sample was mixed with 60 μL of Ag-Path-ID One-Step RT-PCR kit (Applied Biosystems, Foster City, CA) and this mixture was loaded onto the eight ports of the TAC, sealed, and loaded into the ViiATM7 instrument (Applied Biosystems). TAC can detect the following pathogens: bacteria: *Aeromonas, Bacteroides fragilis, Campylobacter* (*C. jejuni* and *C. coli*), *Clostridium difficile*, EAEC, EPEC, ETEC, *Helicobacter pylori*, *Salmonella, Shigella/EIEC, STEC*, and *Vibrio cholerae*, fungi: *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi*, nematodes: *Ancylostoma duodenale*, *Ascars lumbricoides*, *Necator americanus*, *Strongyloides stercoralis*, and *Trichuris trichiura*, protozoan parasites: *Cryptosporidium, Cyclospora, Entameoba histolytica, Giardia A/B*, and *Isospora* and viruses: adenovirus, astrovirus, norovirus GI/GII, rotavirus, and sapovirus [23]. Analysis of raw data files were processed using ViiATM7 software version 1.2.2 (Applied Biosystems) as previously described [23]. A threshold cycle (Ct) greater than 35 was used as the analytical cutoff (lower limit of detection).

**Statistical methods**
Statistical analysis was conducted using IBM SPSS Statistics version 24.0. Chi squared tests were used to determine if the association between patient questionnaire data and subsequent pathogen identification was significant.

**Results**
A total of 48 acute diarrhea cases were enrolled from 2013 to 2017 (47 completed questionnaire), with 9 cases in 2013, followed by 8 (2014), 18 (2015), 9 (2016) and 4 (2017) cases respectively. 38% (18/47) of the stool samples were described as “loose”, 21% (10/47) as “soft”, 23% (11/47) as “watery”, 6% (3/47) as “no record” and the remaining as “formed”. 89.4% (42/47) of the subjects consumed locally prepared food. Primary self-reported complaints included: 72% (34/47) abdominal pain, 64% (30/47) nausea and 34% (16/47) vomiting. Bowel movement frequencies varied between 2 and 20 times in 48 h. Seven patients suffered from bloody diarrhea and three of the seven presenting with bloody diarrhea accompanied by nausea, vomiting, abdominal pain, and bowel movement pain. The clinical presenting data has been described on Table 1.

| Year | Total number | Bloody diarrhea (case) | Nausea (case) | Vomiting (case) | Abdominal pain (case) | Bowel movement range (times) |
|------|--------------|------------------------|---------------|----------------|-----------------------|----------------------------|
| 2013 | 9            | 0                      | 7             | 6              | 6                     | 2–10                       |
| 2014 | 8            | 2                      | 7             | 4              | 7                     | 4–20                       |
| 2015 | 18           | 2                      | 10            | 3              | 12                    | 3–20                       |
| 2016 | 9            | 2                      | 5             | 1              | 6                     | 4–20                       |
| 2017 | 4            | 1                      | 1             | 2              | 3                     | 7–20                       |
Pathogen detection for stool samples collected from 2013 and 2017 was performed using ELISAs and TAC. Microbiological culture and multiplex PCR were added to the diagnostic panel for samples collected in 2015–2017. Of the 48 acute diarrhea stool samples, enteric pathogens were identified in 79.2% (38/48) of the samples while an etiologic agent was not detected in 20.8% (10/48) of the stool samples. The pathogenic profile of the 48 study samples from 2013 to 2017 is summarized in Table 2. Briefly, the most common detected pathogen was *Campylobacter* spp. (43.8%; 21/48), followed by the diarrheagenic *E. coli* (42%; 20/48) and *Salmonella* spp. (23%; 11/48). The most common *Campylobacter* species was *C. jejuni* at 25% (12/48), whereas *Campylobacter* spp. was identified in 18.8% (9/48) of the samples. Of the twenty diarrheagenic *E. coli* cases, 65% (13/20) were EPEC, followed by 15% (3/20) ETEC, 15% (3/20) EAEC, and 5% (1/20 EIEC). *Salmonella* spp. and norovirus were detected in 23% (11/48) and 15% (7/48) of the stool samples respectively (Table 2). Additionally, *Vibrio cholera* and *V. parahaemolyticus* were detected in US military personnel stationed at the Chonburi province, a coastal city, in 2016.

Co-infections (defined as more than one etiologic agent) were detected in 46% (22/48) of the study samples with one sample from 2016 containing seven enteric pathogens: *Aeromonas veronii* bv *sobria*, *Arcobacter butzleri*, *C. jejuni*, EPEC, *Plesiomonas shigelloides*, *V. cholera*, and *V. parahaemolyticus*. Stool samples containing the diarrheagenic *E. coli* and *P. shigelloides* were found to be most commonly associated with those US service members who were exposed to poultry (*p = 0.02*). One surprising observation was the absence of any typical etiologic agents for 2/10 samples that were classified as bloody diarrhea.

All pathogenic bacterial isolates obtained from 2015 to 2017 were further sub-cultured to perform AST, with resulting antibiotic resistance profiles contained in Table 3. 100% of the *Salmonella* isolates were resistant to AMP and 44.4% resistant to SXT. 52.9% (9/17) of the diarrheagenic *E. coli* isolates were resistant to AMP and 100% (4/4) of the EPEC isolates resistant to SXT. One *C. jejuni* and one *A. butzleri* isolate were resistant to AZM. The AZM-resistant *C. jejuni* was also resistant to ERY. 100% of the *C. jejuni* isolates were resistant to CIP and NA. All of the *Plesiomonas* and *Aeromonas* isolates were susceptible to all tested antibiotics.

### Discussion

Diarrhea remains a leading cause of acute morbidity and chronic health effects, negatively impacting the health and functionality of both traveler and military populations. US military service members often deploy into developing regions in which enteric pathogens associated with diarrheal disease are prevalent. *Campylobacter* was the most frequent pathogen identified in this study, which correlates to the high prevalence in travelers with acute diarrhea in previous studies in Thailand in travelers and US military service members participating in previous Cobra Gold exercises [5, 24, 25]. *Campylobacter* isolates from this study were also entirely resistant to quinolones (NA) and fluoroquinolone (CIP) antibiotics, which is of additional concern based upon recent evidence indicating that quinolone- and fluoroquinolone-resistant *Campylobacter* infections are associated with the development of post-infectious long term sequelae to include Guillen Barre Syndrome [26, 27]. *A. butzleri*, a member of the *Campylobacteraceae* family, was isolated from one stool samples. *Arcobacter* species are not typically associated with diarrheal disease, however, previous studies showed an 8% prevalence of traveler’s diarrhea

### Table 2 Summary of identified pathogens present in stool samples collected during 2013 to 2017 from US military personnel presenting with diarrheal disease while deployed in Thailand

| Year          | Pathogen detection | Detection rate (percent) |
|---------------|--------------------|--------------------------|
| *Campylobacter* | *Campylobacter jejuni* | 12/48 (25%) |
|               | *Campylobacter species* | 9/48 (18.8%) |
|               | Total | 21/48 (43.8%) |
| Diarrheagenic *E. coli* | EPEC | 13/48 (27%) |
|               | ETEC | 3/48 (6%) |
|               | EAEC | 3/48 (6%) |
|               | EIEC | 1/48 (2%) |
|               | Total | 20/48 (42%) |
| *Salmonella spp.* | *Salmonella group B* | 5/48 (10%) |
|               | *Salmonella group c* | 4/48 (8%) |
|               | *Salmonella species* | 2/48 (4%) |
|               | Total | 11/48 (23%) |
| *Norovirus* | Norovirus GI | 5/48 (10%) |
|               | Norovirus GII | 2/48 (4%) |
|               | Total | 7/48 (15%) |
| *Plesiomonas shigelloides* | | 6/48 (13%) |
| *Aeromonas species* | | 6/48 (13%) |
| *Vibrio species* | | 3/48 (6%) |
| *Rotavirus* | | 2/48 (4%) |
| *Helicobacter pylori* | | 2/48 (4%) |
| *Shigella species* | | 1/48 (2%) |
| *Arcobacter butzleri* | | 1/48 (2%) |
| *No pathogen detected* | | 10/48 (21%) |

*Diarrheagenic E. coli* [EPEC = Enteropathogenic *E. coli*, ETEC = Enteroaggregative *E. coli*, EAEC = Enteroinvasive *E. coli*]
associated with *A. butzleri* in Mexico, Guatemala, and India [28]. Study of tourist restaurants in Thailand suggested that *Arcobacter* was a food-borne pathogen and its isolates were frequently resistant to AZM which is the common therapeutic recommendation for the treatment of diarrhea in Asia [29]. The AZM-resistant *C. jejuni* was also resistant to ERY, the recommended antimicrobial treatment in invasive cases or to eliminate carrier states. Erythromycin resistance has been reported in Thailand previously [30].

The second most common etiologic diarrhea agent identified in this study was *E. coli*. ETEC is the leading cause of childhood diarrhea and the most frequent cause of diarrhea in travelers to developing countries [31]. ETEC contribution to diarrheal disease is dependent upon the region of interest and seasonality [32–34]. In this study, EPEC was detected more commonly in cases than ETEC. A previous study noted that EPEC was dependent upon co-infection with other pathogenic bacteria to include *Aeromonas* and *Salmonella* in travelers who developed travelers’ diarrhea [35]. However in our study, EPEC was detected in only one co-infected sample. Non-typhoidal *Salmonella* (NTS) was the third most common pathogen detected and associated with travelers’ diarrhea though this study indicated that these pathogens were detected in samples with other enteric pathogens. These co-infection results, associated with clinical diarrhea in military patients, support evidence from previous studies that *Aeromonas* contribute towards the development of diarrhea [39]. Meng et al. reported that synergy or antagonism among pathogens likely affected the degree of diarrheal disease severity more than a single infection in children [40], and that the presence of multiple infections dramatically challenged the ability to properly identify the actual etiological agents of diarrhea disease.

There were several limitations to the study. A relatively small number of diarrheal stool samples were collected with no matched control sample which makes stating that the identified pathogen(s) were truly the cause of the diarrhea. Another limitation is the lack of antibiotic profiles for the bacterial pathogens detected in samples from 2013 to 2014 as the main diagnostic methodology used in these years were ELISAs and TAC. Inclusion of conventional microbiological methods allowed for the determination of antibiotic susceptibility profiles. Due to diagnostic limitations, some pathogens remain undetectable by these methods because they require challenging or unknown unfavorable growth conditions. A previous study indicates that *C. consisus* and *C. ureolyticus* are emergent-bacterial diarrheal pathogens [41]. However, these organisms are obligate anaerobes that require a H2-enriched atmosphere for optimum growth [42]. Methods to identify these pathogens were not used in this study.

### Conclusions

Ongoing diarrheal etiologic agent surveillance studies with antibiotic susceptibility testing should continue in large scale US military exercises these studies relay

---

**Table 3** Antibiotic susceptibility profile of enteric bacteria detected in stool samples from US military personnel presenting with diarrheal disease while deployed to Thailand from 2015–2017

| Identified bacteria | Total isolates | Resistant isolates | Isolates with antibiotic resistance* |
|---------------------|---------------|-------------------|-------------------------------------|
|                     |               | AMP   | AZM   | ERY  | CIP  | NA   | SXT  | CRO  |
| Campylobacter jejuni| 11            | 11    | –     | 1    | 1    | 11   | 11   | –    |
| Arcobacter butzleri | 1             | 1     | –     | 1    | 0    | 0    | 0    | –    |
| Salmonella species  | 9             | 9     | 9     | –    | –    | 0    | 4    | 0    |
| ETEC                | 3             | 2     | 2     | –    | –    | 0    | –    | 0    |
| EAEC                | 3             | 3     | 3     | –    | –    | 0    | –    | 2    |
| EPEC                | 11            | 4     | 4     | –    | –    | 1    | 4    | 0    |
| Shigella sonnei     | 1             | 1     | 0     | –    | –    | 0    | –    | 1    |
| Vibrio species      | 3             | 2     | 1     | –    | –    | 0    | –    | 0    |

*AMP ampicillin, AZM azithromycin, ERY erythromycin, CIP ciprofloxacin, NA nalidixic acid, SXT trimethoprim/sulfamethoxazole, CRO ceftriaxone

---
critical information necessary to protect traveler and military populations and to minimize diarrheal disease threats.

Acknowledgements
The study is supported by the Armed Forces Health Surveillance Branch (AFHSB) and the Global Emerging Infectious Disease Surveillance (GEIS) Response Section.

Authors’ disclaimers
Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Authors’ contributions
WL participated in the conception and design of the study. WL, OS, PL, and NR performed the laboratory work. AS, WO and NK were study site coordinators and management. WL, BAV, STD, WN, JMC and BES analyzed the data, wrote the manuscript, contributed to the analysis and helped in writing the manuscript. All authors read and approved the final manuscript.

Funding
The study is supported by the Armed Forces Health Surveillance Branch (AFHSB) and it’s GEIS (Global Emerging Infectious Disease Surveillance and Response) Section.

Availability of data and materials
Data sharing not applicable to this article.

Ethics approval and consent to participate
This study protocol was not qualify as research in accordance with Walter Reed Army Institute of Research (WRAIR) Policy Letter#12-09, as the work described involves United State Army-mandated (Force Health protection) public health surveillance.

Consent for publication
Not applicable.

Completing interests
The authors declare that there is no completing interests.

Author details
1 Department of Bacterial and Parasitic Diseases, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. 2 Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, USA.

Received: 16 January 2020 Accepted: 4 April 2020 Published online: 09 April 2020

References
1. Connor P, Porter CK, Swierczewski B, Riddle MS. Diarrhoea during military deployment: current concepts and future directions. Curr Opin Infect Dis. 2012;25(5):546–54.
2. Riddle MS, Tribble DR, Putnam SD, Mostafa M, Brown TR, Letizia A, et al. Past trends and current status of self-reported incidence and impact of disease and nonbattle injury in military operations in Southwest Asia and the Middle East. Am J Public Health. 2008;98(12):2199–206.
3. Diemert DJ. Prevention and self-treatment of traveler’s diarrhea. Clin Microbial Rev. 2006;19(3):583–94.
4. Dupont AW, Dupont HL. Traveler’s diarrhea: modern concepts and new developments. Curr Treat Options Gastroenterol. 2006;9(1):13–21.
5. Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers’ diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg. 2009;80(4):609–14.
6. Aw B, Boraston S, Botten D, Cherniwchan D, Fazal H, Kelton T, et al. Travel medicine: what’s involved? when to refer? Can Fam Physician. 2014;60(12):1091–103.
7. Jiang ZD, Lowie B, Verenikin MP, Ashley D, Steffen R, Torneiporth N, et al. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). J Infect Dis. 2002;185(4):497–502.
8. Riddle MS, Sanders JW, Putnam SD, Tribble DR. Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review. Am J Trop Med Hyg. 2006;74(S5):891–900.
9. Echeverria P, Jackson LR, Hoge CW, Arness MK, Dunnavant GR, Larsen RR. Diarrhea in U.S. troops deployed to Thailand. J Clin Microbiol. 1993;31(12):3531–2.
10. Beecham HJ 3rd, Lebron CI, Echeverria P. Short report: impact of travelers’ diarrhea on United States troops deployed to Thailand. Am J Trop Med Hyg. 1997;57:699–701.
11. Sanders JW, Isenbarger DW, Walz LW, Scott DA, Tammenga C, et al. An observational clinic-based study of diarrheal illness in deployed United States military personnel in Thailand: presentation and outcome of Campylobacter infection. Am J Trop Med Hyg. 2002;67(5):533–8.
12. James HJ, Michael AP, Karen CC, Guido FMLL, Sandry SR, David WW. Manual of clinical microbiology. Washington DC: American Society for Microbiology press; 2015.
13. Clinical and Laboratory Standards Institute. Performace standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. CLSI document M100-S25. Wayne: Clinical and Laboratory Standards Institute; 2015. p. 231.
14. National Center for Emerging and Zoonotic Infectious Diseases. Division of foodborne, waterborne and environmental diseases. Enteric bacteria, human isolates final report. Atlanta: National Antimicrobial Resistance Monitoring System (NARMS); 2013.
15. Karch H, Bohm H, Schmidt H, Gunzer F, Aleksic S, Heesemann J. Clonal structure and pathogenicity of Shiga-like toxin-producing, sorbitol-fermenting Escherichia coli D157. J Clin Microbiol. 1993;31(5):1200–5.
16. Tompieporth NG, John J, Salgado K, de Jesus P, Latham E, Melo MC, et al. Differentiation of pathogenic Escherichia coli strains in Brazilian children by PCR. J Clin Microbiol. 1995;33(5):1371–4.
17. Boisen N, Struve C, Scheutz F, Kroghfelt KA, Nataro JP. New adhesion of enteroaggregative Escherichia coli related to the Afa/DraA family. Infect immune. 2008;76(7):5281–92.
18. Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H. Development of PCR for screening of enteroaggregative Escherichia coli. J Clin Microbiol. 1995;33(3):701–5.
19. Bolin L, Wiklund G, Qadri F, Torres O, Bourgeois AL, Savarino S, et al. Enterotoxigenic Escherichia coli with 5Th and 5Tp genotypes is associated with diarrhea both in children in areas of endemicity and in travelers. J Clin Microbiol. 2006;44(11):3872–7.
20. Luscher D, Altweg M. Detection of shigellae, enteroinvasive and enterotoxigenic Escherichia coli using the polymerase chain reaction (PCR) in patients returning from tropical countries. Mol Cell Probes. 1994;8(4):285–90.
21. Rodas C, Iniguez V, Qadri F, Wiklund G, Svennerholm AM, Sjoling A. Development of multiplex PCR assays for detection of enteroaggregative Escherichia coli colonization factors and toxins. J Clin Microbiol. 2009;47(4):1218–20.
22. Vidal R, Vidal M, Lagos R, Levine M, Prado V. Multiplex PCR for diagnosis of enteric infections associated with diarrheagenic Escherichia coli. J Clin Microbiol. 2004;42(4):1787–9.
23. Lertsethtakarn P, NaKaron K, Silapong S, Neesanant P, Sakpaisal P, et al. Differentiation of pathogenic Escherichia coli strains in Brazilian children by PCR. J Clin Microbiol. 1995;33(3):701–5.
24. Bolin L, Wiklund G, Qadri F, Torres O, Bourgeois AL, Savarino S, et al. Enterotoxigenic Escherichia coli with 5Th and 5Tp genotypes is associated with diarrhea both in children in areas of endemicity and in travelers. J Clin Microbiol. 2006;44(11):3872–7.
25. Luscher D, Altweg M. Detection of shigellae, enteroinvasive and enterotoxigenic Escherichia coli using the polymerase chain reaction (PCR) in patients returning from tropical countries. Mol Cell Probes. 1994;8(4):285–90.
26. Mølbak K. Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. Clin Infect Dis. 2005;41(11):1613–20.
27. Olson S, Hall A, Riddle MS, Porter CK. Travelers’ diarrhea: update on the incidence, etiology and risk in military and similar populations 1990–2005 versus 2005–2015; does a decade make a difference? Trop Dis Travel Med Vaccines. 2019;15(1):1.
28. Jiang ZD, DuPont HL, Brown EL, Nandy RK, Ramamurthy T, Sinha A, et al. Microbial etiology of travelers’ diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic Bacteroides fragilis and Arcobacter species. J Clin Microbiol. 2010;48(4):1417–9.
29. Teague NS, Srijan A, Wongstitwilairoong B, Poramathikul K, Champathai T, Ruksasiri S, et al. Enteric pathogen sampling of tourist restaurants in Bangkok, Thailand. J Travel Med. 2010;17(2):118–23.
30. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates. Emerg Infect Dis. 2001;7(1):24–34.
31. Ahn JY, Chung JW, Chang KJ, You MH, Chai JS, Kang YA, et al. Clinical Characteristics and etiology of travelers’ diarrhea among Korean travelers visiting South-East Asia. J Korean Med Sci. 2011;26(2):196–200.
32. Ericsson CD, DuPont HL. Travellers’ diarrhoea: approaches to prevention and treatment. Clin Infect Dis. 1993;16(5):616–24.
33. Jertborn M, Svennerholm AM. Enterotoxin-producing bacteria isolated from Swedish travellers with diarrhoea. Scand J Infect Dis. 1991;23(4):473–9.
34. Mattila L, Sitonen A, Kyvärinenpää H, Simula J, Oksanen P, Stenvik M, et al. Seasonal variation in etiology of travelers’ diarrhea. Finnish-Moroccan Study Group. J Infect Dis. 1992;165(2):385–8.
35. Keskimäki M, Mattila L, Peltola H, Sitonen A. Prevalence of diarrheagenic Escherichia coli in Finns with or without diarrhea during a Round-the-World Trip. J Clin Microbiol. 2000;38(2):4425–9.
36. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O’Brien SJ, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010;50(6):882–9.
37. Srijan A, Lurchachaiwong W, Wongstitwilairoong B, Bodhidatta L, Mason C, Swierzewski B. Prevalence and antimicrobial resistance of nontyphoid Salmonella in military personnel, 1988–2013. Asian Pac J Trop Med. 2018;11(6):387–92.
38. de la Cabada Bauche J, Dupont HL. New developments in traveler’s diarrhea. Gastroenterol Hepatol (N Y). 2011;7(2):88–95.
39. Figueras MJ, Horneman AJ, Martinez-Murcia A, Guarro J. Controversial data on the association of Aeromonas with diarrhoea in a recent Hong Kong study. J Med Microbiol. 2007;56:996–8.
40. Meng CY, Smith BL, Bodhidatta L, Richard SA, Vansith K, Thy B, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. Pediatr Infect Dis J. 2011;30(4):331–5.
41. Serchantalergs O, Ruekit S, Pandey P, Anura S, Mason C, Bodhidatta L, et al. Incidence of Campylobacter concisus and C. ureolyticus in traveler’s diarrhea cases and asymptomatic controls in Nepal and Thailand. Gut Pathog. 2017;17(9):47.
42. Man S. The clinical importance of emerging Campylobacter species. Nat Rev Gastroenterol Hepatol. 2011;8(12):669–85.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.