# SXT ELEMENT, CLASS 1 INTEGRON AND MULTIDRUG-RESISTANCE GENES OF *VIBRIO CHOLERAE* ISOLATED FROM CLINICAL AND ENVIRONMENTAL SOURCES IN NORTHEAST THAILAND

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**Abstract.** Emergence of multiple drug resistance in *Vibrio cholerae* has been increasing around the world including Northeast Thailand. In this study, 92 isolates of *V. cholerae* (50 O1 and 42 non-O1/non-O139 isolates) from clinical and environmental sources in Northeast Thailand were randomly selected and investigated for the presence of SXT element, class 1 integron and antimicrobial resistance genes. Genotypic-phenotypic concordance of antimicrobial resistance was also determined. Using PCR-based assays, 79% of *V. cholerae* isolates were positive for SXT element, whereas only 1% was positive for class 1 integron. SXT element harbored antimicrobial resistance genes, *dfrA1* or *dfr18*, *floR*, *strB*, *sul2*, and *tetA*. Overall phenotypic-genotypic concordance of antimicrobial resistance was 78%, with highest and lowest value being for trimethoprim (83%) and chloramphenicol (70%), respectively. Ninety-two percent of *V. cholerae* O1 strains isolated from clinical sources harbored both *dfrA1* (O1-specific trimethoprim resistance gene) and *dfr18* (non-O1-specific trimethoprim resistance gene), whereas only 5% of *V. cholerae* non-O1/non-O139 strains harbored both genes. All *V. cholerae* O1 isolated from environmental source harbored *dfr18* but 48% of *V. cholerae* non-O1/non-O139 harbored *dfrA1*. This study indicates that SXT element was the main contributor to the circulation of multiple-drug resistance determinants in *V. cholerae* strains in Northeast Thailand and that genetic exchange of SXT element can occur in both *V. cholerae* O1 and non-O1/non-O139 strains from clinical and environmental sources.

**Keywords:** *Vibrio cholerae*, antimicrobial resistance, class 1 integron, clinical source, environmental source, SXT element

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## INTRODUCTION

Cholera is an infectious disease caused by consumption of food or drinking water contaminated with *Vibrio cholerae* (Ali *et al*, 2011). *V. cholerae* O1 and O139 are the major causative agents of cholera epidemics,

while non-O1 and non-O139 serogroups cause a less severe form of diarrheal disease and have no epidemic potential (Tamrakar *et al*, 2009).

Emergence of multiple drug-resistant *V. cholerae* has been increasing around the world including in Thailand (Chomvarin *et al*, 2013). Acquisition of antimicrobial resistance is attributed to horizontal gene transfer by plasmids, transposons, SXT element and integrons (Kumar *et al*, 2010). SXT element and class 1 integrons have been reported to be associated with rapid spread of antimicrobial resistance genes in *V. cholerae* (Dalsgaard *et al*, 2001; Hochhut *et al*, 2001).

SXT element is an integrative conjugating element (ICE) belonging to the SXT/R391 family. It is a self-transmissible mobile genetic element that can be integrated into bacterial chromosome and be transferred to a new host by conjugation (Ahmed *et al*, 2005; Burrus *et al*, 2006). SXT element confers resistance against many antimicrobial agents, including chloramphenicol (*floR*), streptomycin (*strB*), sulfamethoxazole (*sul2*), trimethoprim (*dfrA1*, conferring O1-specific and *dfr18* non-O1-specific resistance), and tetracycline (*tetA*) (Iwanaga *et al*, 2004; Mohapatra *et al*, 2008). SXT element integrates into and excises from bacterial chromosome using its own integrase (encoded by $int_{SXT}$) (Ahmed *et al*, 2005; Burrus *et al*, 2006). In Thailand, SXT element has been studied only in *Vibrio* spp isolated from environmental sources but not from clinical material (Kitiyodom *et al*, 2010).

Integrons are nonself-transmissible mobile genetic elements that often are transferred via transposons or conjugative plasmids. They provide natural genetic engineering platforms for incorporating gene cassettes into bacterial chromosomes and converting them into functional genes (Dalsgaard *et al*, 2001). Class 1 integrons are the most frequently found in clinical isolates of gram-negative bacteria including *V. cholerae* (Dalsgaard *et al*, 2000; Dalsgaard *et al*, 2001) and have been identified in *V. cholerae* O1 strains isolated in Vietnam (Dalsgaard *et al*, 1999), Albania and Italy (Falbo *et al*, 1999). Previous studies in Thailand reported that class 1 integrons are more frequent in clinical *V. cholerae* O1 and non-O1/non-O139 than in environmental strains (Dalsgaard *et al*, 2000).

In Northeast Thailand, *V. cholerae* O1 and non-O1/non-O139 first manifested resistance to trimethoprim/sulfamethoxazole and/or tetracycline in early 2007, but levels of resistance declined after 2010 (Chomvarin *et al*, 2013). To date, very little information is available regarding class 1 integrons and SXT element and antimicrobial resistance genes in clinical and environmental isolates of *V. cholerae* in Thailand. In this study, antimicrobial resistance status of *V. cholerae* O1 and non-O1/non-O139 isolates from clinical and environmental sources was investigated for the presence of class 1 integrons and SXT element. Antimicrobial resistance genes were assayed using PCR for the presence of *dfrA1*, *dfr18*, *floR*, *sul2*, *strB* and *tetA*. Phenotypic and genotypic determinations of antimicrobial resistance of *V. cholerae* isolated from clinical and water samples in this region were also compared. This study provides a better insight into the mechanisms of multidrug resistance of *V. cholerae* in environmental and clinical sources and the association between phenotypic and genotypic determinants in this bacterium. The findings in this study should be useful for epidemiological control and monitoring of these multidrug resistant *V. cholerae* strains in Northeast Thailand.

## MATERIALS AND METHODS

### Bacterial strains

Randomly selected *V. cholerae* isolates ($n$ = 92) collected between 2004 and 2012 were investigated. Sixty-seven clinical isolates (48 *V. cholerae* O1 and 19 non- O1/ non- O139) were obtained from hospitals in Khon Kaen, Udon Thani, Nong Khai and Loei in Northeast Thailand, and 25 environmental isolates (2 *V. cholerae* O1 and 23 non-O1/non-O139) were from aquatic areas in Khon Kaen municipality.

### Isolation and identification of *V. cholerae* isolates

Bacterial isolates were identified by culturing on selective media (thiosulfate-citrate-bile salt-sucrose agar (Eiken Chemical, Tokyo, Japan) and identified as *V. cholerae* using standard biochemical tests (Ramamurthy and Nair, 2007) and serotyped using a slide agglutination test with polyvalent antiserum specific to *V. cholerae* O1/O139 and with monovalent antiserum to Ogawa and Inaba (Oxoid, Columbia, MD).

### Antimicrobial susceptibility test

Antimicrobial susceptibility test was determined using a disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2012). All *V. cholerae* isolates were tested for susceptibility to 10 antimicrobial agents (Oxoid, Unipath, Basingstroke, Hamshire, UK): ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (CIP, 5 μg), erythromycin (E, 10 μg), gentamicin (GM, 10 μg), norfloxacin (NOR, 10 μg), streptomycin (S, 10 μg), tetracycline (TE, 30 μg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75). *Escherichia coli* ATCC 25922 was used as a quality control strain. Results are interpreted after incubation at 37°C for 18 hours as susceptible (S), intermediate (I) or resistant (R) according to CLSI (2012) criteria.

### PCR assay

Genomic DNA of *V. cholerae* was extracted using a method modified from a previous study (Ahmed *et al*, 2005). In brief, a 200 μl aliquot of overnight bacterial culture was centrifuged at 14,000*g* for 5 minutes. The pellet was resuspended in sterile distilled water, boiled for 10 minutes, cooled on ice, centrifuged at 2,000*g* for 5 minutes, and supernatant used as template for PCR. Specific primers, PCR conditions and expected amplicon sizes for detection of *V. cholerae* class 1 integrons, SXT element and specific antimicrobial resistance genes are shown in Table 1. The amplification reactions were conducted in a 25-μl reaction mixture. Thermocycling was conducted in a Bio-Rad C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Amplicons were analyzed by 1.5% agarose gel-electrophoresis, stained with ethidium bromide and visualized under a UV light transilluminator (Bio-Rad Gel™ Doc XR+ Imager).

## RESULTS

The majority (79%) of *V. cholerae* isolates exhibited multiple drug resistance phenotypes (Table 2). PCR-based assays of the 92 isolates for the presence of class 1 integron, SXT element and antimicrobial resistance genes, namely, *dfrA1* and *dfr18* (conferring trimethoprim resistance), *floR* (chloramphenicol resistance), *strB* (streptomycin resistance), *sul2* (sulfamethoxazole resistance), and *tetA* (tetracycline resistance) (Fig 1). Seventy-two (79%) *V. cholerae* isolates were positive for $int_{SXT}$, but only one environmental isolate was positive for class 1 integron (Table 2).

The overall concordance between

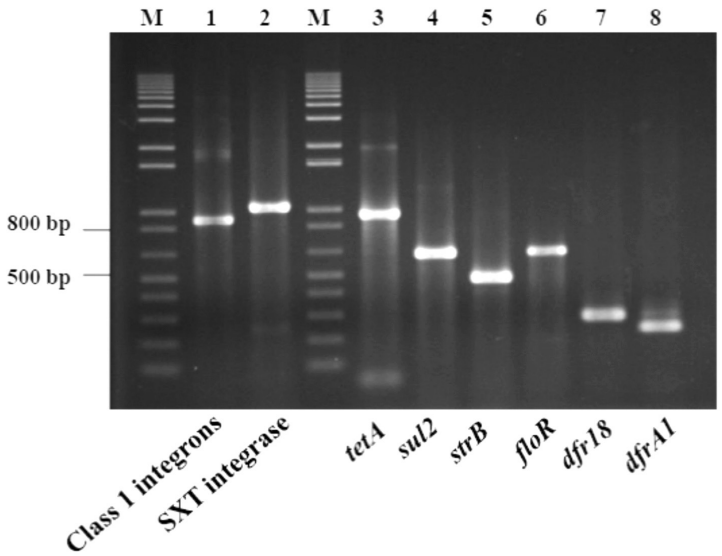


Fig 1–Gel-electrophoregram of amplicons of class 1 integrons, SXT element and antimicrobial resistance genes. *Vibrio cholerae* DNA were amplified with target gene-specific primers, and analyzed by 1.5% agarose gel-electrophoresis. Lane M, 1 kbp DNA size markers; lane 1, class 1 integron (*int1*); lane 2, SXT integrase gene ($int_{SXT}$); lane 3, tetracycline resistance gene (*tetA*); lane 4, sulfamethoxazole resistance gene (*sul2*); lane 5, streptomycin resistance gene (*strB*); lane 6, chloramphenicol resistance gene (*floR*); lanes 7, trimethoprim resistance gene of *V. cholerae* non-O1 (*dfr18*); lane 8, trimethoprim resistance gene of *V. cholerae* O1 (*dfrA1*).

the presence of resistance genes and resistance phenotype based on agar disk diffusion was 78% (Table 2). Concordance between individual resistance gene and phenotype ranged from 70% (chloramphenicol resistance) to 83% (trimethoprim resistance) (Table 2).

Both trimethoprim resistance genes, *dfrA1* (specific to *V. cholerae* O1) and *dfr18* (specific to *V. cholerae* non-O1/non-O139) were most frequent (92%) in *V. cholerae* O1 isolated from clinical source and 8% of these strains harbored only *dfrA1* (Table 3). On the other hand the majority (53%) of *V. cholerae* non-O1/non-O139 isolated from clinical source harbored only *dfr18* and only 5% of those harbored both genes, indicating that *V. cholerae* O1 from clinical source had a higher frequency of genetic exchange than *V. cholerae* non-O1/non-O139. All *V. cholerae* O1 isolated from environmental source harbored *dfr18* but 48% of *V. cholerae* non-O1/non-O139 harbored *dfrA1*. The results indicate that there was also a high frequency of genetic exchange of resistance genes among *V. cholerae* in the environment.

## DISCUSSION

In Northeast Thailand, *V. cholerae* O1 and non-O1/non-O139 strains have exhibited increasing resistance to trimethroprim/sulfamethoxazole and tetracycline since 2007 (Chomvarin *et al*, 2013). Previous studies showed that class 1 integrons were associated with the dissemination of genetic determinants of resistance to antimicrobial agents in both clinical and environmental sources in Thailand (Dalsgaard *et al*, 2000; Kitiyodom *et al*, 2010). However, SXT element has been studied only in *Vibrio* spp isolated from environmental sources but has not been examined in those clinical sources (Kitiyodom *et al*, 2010).

Multiple drug resistance in *V. cholerae* is caused by horizontal transfer of resistance genes and by the action of multiple

Table 1
Specific primers, PCR conditions and expected amplicon for detection of class 1 integron, SXT element and antimicrobial resistant genes in *Vibrio cholerae*.

| Target gene | Amplicon (bp) | Function | Primer sequence | PCR conditions | Reference |
|---|---|---|---|---|---|
| *int1* | 923 | Class 1 integron | F-5′- GTTCGGTCAAGGTTCTG-3′<br>R-5′- GCCAACTTTCAGCACATG-3′ | 94°C, 30 sec; 50°C, 30 sec; 72°C, 1.30 min (35 cycles) | (Shi *et al*, 2006) |
| $int_{SXT}$ | 1,035 | SXT integrase | F-5′- ATGGCGTTATCAGTTAGCTGGC-3′<br>R-5′- GCGAAGATCATGCATAGACC-3′ | 94°C, 1 min; 60.5°C, 1 min; 72°C, 1 min (35 cycles) | (Bhanumathi *et al*, 2003) |
| *tetA* | 950 | Tetracycline resistance | F-5′- GTAATTCTGAGCACTGTCGC-3′<br>F-5′- CTGCCTGGACAACATTGCTT-3′ | | (Schmidt *et al*, 2001) |
| *sul2* | 623 | Sulfamethoxazole resistance | F-5′-TGCGGATGAAGTCAGCTCC-3′<br>R-5′-GGGGGCAGATGTGATCGAC-3′ | 94°C, 30 sec; 57°C, 1 min; 72°C, 2 min (35 cycles) | Modified from (Hochhut *et al*, 2001) |
| *dfrA1* | 278 | Trimethoprim resistance (*V. cholerae* O1) | F-5′- CAAGTTTACATCTGACAATGAGAACGTAT-3′<br>R-5′- ACCCTTTTGCCAGATTTGGTA -3′ | | (Falbo *et al*, 1999) |
| *dfr18* | 389 | Trimethoprim resistance (*V. cholerae* non-O1) | F-5′-ACTGCCGTTTTCGATAATGTGG-3′<br>R-5′-TGGGTAAGACACTCGTCATGGG-3′ | | (Hochhut *et al*, 2001) |
| *strB* | 470 | Streptomycin resistance | F-5′- GGCAGCATCAGCCTTATAATTT-3′<br>R-5′-GTGGATCCGTCATTCATTGTT-3′ | | (Ramachandran *et al*, 2007) |
| *floR* | 526 | Chloramphenicol resistance | F-5′- TTATCTCCCTGTCGTTCCAGCG-3′<br>R-5′- CCTATGAGCACACGGGGAGC-3′ | | (Iwanaga *et al*, 2004) |

Table 2
Association of phenotypic with genotypic antimicrobial resistance of *V. cholerae* isolates from clinical and environmental sources.

| Sources of *V. cholerae* (No. of strains) | Antimicrobial resistance profile | Number of isolates | $int_{SXT}$ | Concordance between phenotype and genotype | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | SXT/*sul2* | TMP/*dfrA1* or *dfr18* | S/*strB* | C/*floR* | TE/*tetA* |
| **Clinical (67)** | | | | | | | | |
| O1 (48) | SXT, TE, S, AMP | 2 | + | +/+ | +/+ | +/+ | -/+ | +/+ |
| | SXT, TE, S, E | 1 | + | +/+ | +/+ | +/+ | -/- | +/+ |
| | SXT, TE, S | 30 | + | +/+ | +/+ | +/+ | -/- | +/+ |
| | | 4 | + | +/+ | +/+ | +/+ | -/- | +/- |
| | | 2 | + | +/+ | +/+ | +/+ | -/+ | +/+ |
| | | 1 | + | +/+ | +/+ | +/+ | -/- | -/- |
| | | 1 | + | +/+ | +/+ | +/+ | -/+ | +/- |
| | SXT, S, C | 2 | + | +/+ | +/+ | +/+ | +/+ | -/+ |
| | SXT, S | 1 | + | +/+ | +/+ | +/+ | -/- | -/+ |
| | | 1 | + | +/- | +/+ | +/+ | -/- | -/+ |
| | S, AMP | 1 | - | -/- | -/+ | +/+ | -/- | -/- |
| | S, E | 1 | - | -/- | -/+ | +/+ | -/- | -/- |
| | S, NOR | 1 | - | -/- | -/+ | +/- | -/- | -/- |
| non-O1/ | SXT, TE, S | 7 | + | +/+ | +/+ | +/+ | -/+ | +/+ |
| non-O139 (19) | TE, S, AMP | 1 | - | -/- | -/- | +/+ | -/+ | +/+ |
| | SXT, S | 2 | + | +/+ | +/+ | +/+ | -/+ | -/+ |
| | | 1 | + | +/+ | +/+ | +/+ | -/+ | -/- |
| | SXT | 1 | + | +/+ | +/+ | -/+ | -/+ | -/+ |
| | | 1 | + | +/- | +/+ | -/+ | -/+ | -/+ |
| | S, AMP | 1 | - | -/+ | -/+ | +/- | -/- | -/- |
| | S, E | 1 | - | -/+ | -/+ | +/- | -/- | -/- |
| | | 1 | - | -/- | -/+ | +/- | -/- | -/- |
| | S | 1 | - | -/- | -/- | +/- | -/- | -/- |
| | | 1 | - | -/+ | -/- | +/- | -/- | -/- |
| | | 1 | - | -/- | -/+ | +/- | -/- | -/- |
| **Environmental (25)** | | | | | | | | |
| O1 (2) | SXT, S | 1 | + | +/+ | +/+ | +/+ | -/- | -/- |
| | S | 1 | + | -/+ | -/+ | +/+ | -/- | -/- |
| non-O1/ | SXT, S | 6 | + | +/+ | +/+ | +/+ | -/+ | -/+ |
| non-O139 (23) | SXT | 1* | + | +/- | +/+ | -/+ | -/+ | -/+ |
| | S | 1 | + | -/+ | -/+ | +/+ | -/+ | -/- |
| | | 2 | + | -/+ | -/+ | +/+ | -/- | -/- |
| | | 1 | + | -/+ | -/- | +/+ | -/+ | -/+ |
| | | 1 | + | -/+ | -/- | +/+ | -/+ | -/- |
| | | 1 | + | -/+ | -/- | +/+ | -/- | -/- |
| | | 1 | + | -/+ | -/- | +/+ | -/- | -/+ |
| | | 1 | - | -/- | -/+ | +/+ | -/- | -/- |
| | | 4 | - | -/- | -/+ | +/- | -/- | -/- |
| | | 1 | - | -/- | -/- | +/- | -/- | -/- |
| | | 2 | - | -/+ | -/- | +/- | -/- | -/- |
| | | 1 | - | -/+ | -/- | +/+ | -/- | -/- |
| Total | | 92 | 79% (73/92) | 81% (75/92) | 83% (76/92) | 81% (75/92) | 70% (64/92) | 76% (70/92) |
| | | | | Correlation average = 78% | | | | |

*Positive for class 1 integron. C, chloramphenicol; S, streptomycin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; TMP, trimethoprim. *dfrA1*, trimethoprim resistance gene specific for *V. cholerae* O1; *dfr18*, trimethoprim resistance gene specific for *V. cholerae* non-O1.

Table 3
Prevalence of trimethoprim resistance genes in *V. cholerae* O1 and non-O1/non-O139.

| Trimethoprim resistance gene | | Number of *V. cholerae* O1 isolates (total $n = 50$) | | Number of *V. cholerae* non-O1/non-O139 isolates (total $n = 42$) | |
|---|---|---|---|---|---|
| *dfrA1* (O1) | *dfr18* (non-O1) | Clinical (48) | Environmental (2) | Clinical (19) | Environmental (23) |
| + | + | 44 (92%) | 1 (50%) | 1 (5%) | 6 (26%) |
| + | - | 4 (8%) | 0 | 5 (26%) | 5 (22%) |
| - | + | 0 | 1 (50%) | 10 (53%) | 5 (22%) |
| - | - | 0 | 0 | 3 (16%) | 7 (30%) |

drug efflux pumps (Kitaoka *et al*, 2011; Bhattacharya *et al*, 2012). However, horizontal gene transfer is the most common mechanism mediated by SXT element (Hochhut *et al*, 2001; Kumar *et al*, 2010). This element mediates the transfer of mobilizable plasmids and segments of chromosomal DNA (Hochhut *et al*, 2000). SXT element has been found in *V. cholerae* from many Asian and African countries (Dalsgaard *et al*, 2001; Mohapatra *et al*, 2008). In Thailand, from a total of 83 *Vibrio* isolates, belonging to several species isolated from farmed marine shrimps (*Penaeus monodon*), SXT element was detected in 8% of the isolates. Among these were three *V. cholerae* isolates and class 1 integrons were found only in one isolate of *V. cholerae* (Kitiyodom *et al*, 2010).

In this study, 79% of *V. cholerae* isolates exhibited drug resistance conferred by the SXT element and only one (environmental) isolate harbored class 1 integron. Our results are at variance with those of Dalsgaard *et al* (2000) who reported that class 1 integrons were present among 65% of clinical *V. cholerae* O1 isolates from patients at the provincial hospital in Samut Sakhon, one of the central provinces in Thailand, 13% among *V. cholerae* non-O1/non-O139 isolates from children with diarrhea in Bangkok and 53% of *V. cholerae* non-O1/non-O139 from patients in Aranyaprathet District in Sa Kaeo Province, eastern Thailand. Such discordant results might be explained by temporal and geographic differences. Isolates in our study were collected later, between 2004 and 2012, and from a different part of Thailand. Notably, our findings are in agreement with a recent report from India showing that SXT element is present in 98% of clinical isolates of *V. cholerae* O1 (Kutar *et al*, 2013). However, class 1 integron, SXT element and plasmids bearing drug resistance markers are distributed fairly widely in non-O1, non-O139 strains of *V. cholerae* in India (Thungapathra *et al*, 2002; Mohapatra *et al*, 2008).

We found a high concordance between resistance phenotype and presence of SXT element and associated antimicrobial resistance gene. However, some *V. cholerae* strains carried antimicrobial resistance genes but did not exhibit the corresponding drug-resistant phenotype. This might be explained by a low-level expression of the resistance genes, possible presence of mutation(s) in a resistance gene affecting function of encoded protein, and/or acquisition of incompatible resistance genes from other bacteria (Dantas and Som-

mer, 2012). Conversely, some *V. cholerae* strains showed phenotypic resistance but harbored no resistance gene. This might be explained by resistance conferred by other mechanisms (drug efflux pump) or by other types of drug resistance genes not amplified by our primer sets (Ghosh and Ramamurthy, 2011; Kitaoka *et al*, 2011).

Previous studies showed that *floR*, *sul2*, *strB,* and *tetA* can be transferred by conjugative plasmids (Poole, 2005; Kitaoka *et al*, 2011; Folster *et al*, 2014). We found that 21% of *V. cholerae* isolates did not possess SXT element and yet showed resistance to streptomycin. It is possible that the gene conferring resistance to streptomycin was located on a plasmid (Kitaoka *et al*, 2011; Folster *et al*, 2014), but this was not determined in our study.

Iwanaga *et al* (2004) found that 8% of clinical isolates of *V. cholerae* O1 in Lao PDR harbored *floR*, *strAB*, *sul2*, and *tetA*, but not *dfrA1* or *dfr18*. In India, all clinical isolates of *V. cholerae* O1 also carried genes conferring resistance to sulfamethoxazole, streptomycin and trimethoprim, but not *floR* (Goel *et al*, 2010). Interestingly, we found that *dfrA1* and/or *dfr18* were present in both *V. cholerae* O1 and non-O1/non-O139 isolates from clinical and from environmental samples. The majority of clinical *V. cholerae* O1 isolates harbored both *dfrA1* and *dfr18*, indicating that *V. cholerae* in a clinical setting frequently transfers resistance genes among O1 and non-O1/non-O139 strains. In addition, we found that a high proportion of *V. cholerae* isolates from environmental source harbored both types of *dfr* (50% for *V. cholerae* O1 and 26% for *V. cholerae* non-O1/non-O139) indicating the existence of similar genetic exchanges. The *dfrA1* and *dfr18* found among *V. cholerae* O1 and non-O1/non-O139 in clinical and environmental sources might indicate genetic exchanges of SXT element that can be found in both serogroup and other sources. Such high rates of genetic exchange might influence the spread of antimicrobial resistance or epidemiological and environmental responses (Mohapatra *et al*, 2010; Okoh and Igbinosa, 2010).

In summary, SXT element was the main contributor to the transfer of antimicrobial resistance genes associated with multidrug-resistance of *V. cholerae* in Northeast Thailand. These findings will help us to understand the mechanisms producing drug resistance and facilitate the monitoring of multidrug-resistant *V. cholerae* from environmental as well as clinical sources.

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