Integron Analysis of Multidrug-Resistant Diarrheagenic *E. coli* Isolates from Travelers in Thailand

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Abstract

Multidrug resistant (MDR) diarrheagenic *Escherichia coli* (DEC) is an important agent of traveler's diarrhea. However, the reports on genetic mechanism conferring antimicrobial resistance among MDR-DEC of travelers in Thailand are limited. The aim of this study was to determine the prevalence and characterization of class 1 integrons among 49 MDR-DEC isolates from international travelers in Thailand in 2001-2004 and 2010. The majority (85%) of the isolates exhibited resistance to tetracycline, sulfisoxazole, ampicillin, streptomycin, and trimethoprim/sulfamethoxazole in both travelers with diarrhea and non-diarrhea. The most frequent phenotype was resistant to ampicillin-streptomycin-sulfisoxazole-trimethoprim/sulfamethoxazole-tetracycline. Using PCR specific primers to class 1 integrase (*intI1*) gene, 5′- and 3′-conserved sequences (5′CS-3′CS), and dot-blot hybridization, 37 (75.5%) isolates harbored class 1 integrons, which were high prevalent in both of isolates from diarrhea and non-diarrhea groups. Resistance gene cassettes identified in this study were *aadA1* and *aadA2* (for streptomycin resistance) and *linF* (for lincosamide resistance). The presence of class 1 integrons carrying *aadA2-linF* is described for the first time from clinical sample in Thailand. This study emphasized an important role of class 1 integrons dissemination among community acquired MDR-DEC isolates in Thailand during the past 10 years and suggested the circulation of these resistance elements in this community. Therefore, further studies of the genetic and epidemiology of these organisms will be relevant for public health to limit the emergence of bacterial resistance.

Keywords: Multidrug resistant (MDR), Diarrheagenic *Escherichia coli* (DEC), Integron, Resistance gene cassette

Introduction

Traveler's diarrhea (TD) is a major public health concern causing significant morbidity and disability. TD is considered as individuals travel from industrialized countries to developing regions with low hygiene such as Latin America, the Middle East, South and Southeast Asia, and Africa, and susceptible to enteric infectious agents (de la Cabada Bauche & Dupont, 2011). Certainly, Diarrheagenic *E. coli* (DEC) are one of the most common causes of foodborne diseases in travelers as well as children under five years old in developing countries (Farthing et al., 2013).

The antimicrobial resistance in DEC is an increasing problem (Kalantar, Soheili, Salimi, & Soltan Dallal, 2011; Kargar, Mohammadalipour, Doosti, Lorzadeh, & Japoni-Nejad, 2014; Meng et al., 2011; Mosquito et al., 2012; Nguyen, Le, Le, & Weintraub, 2005); moreover, the global trade and travel provides opportunities for the distribution of antimicrobial resistant bacteria; consequently, a serious threat to public health. For instance, the CTX-M-15-producing enteroaggregative *E. coli* (EAEC) caused diarrhea in travelers who had traveled from Spain to India (Guiral et al., 2011). Horizontal gene transfer plays a major role in bacterial evolution and dissemination of multidrug resistance, since most of resistance genes are located

on mobile genetic elements, e.g., plasmids and transposons. For multidrug resistance, integrons are an important part in the wide spread of multidrug resistance in Gram-negative bacteria (Stalder, Barraud, Casellas, Dagot, & Ploy, 2012). Integrons are genetic elements able to incorporate open reading frames (ORFs) of mobile cassettes carrying antimicrobial resistance genes by site-specific recombination and convert them into functional genes as gene-capturing and expression systems. Integron platform comprises three functional elements necessary for the systemic operation; an integrase encoded by *intI*, which encodes a site-specific tyrosine recombinase, a primary recombination site, *attI*, and a strong promoter, Pc (Cambray, Guerout, & Mazel, 2010). Despite integrons are not self-mobilized, they are usually found in association with mobile genetic elements, e.g., plasmids and transposons, facilitating their spread (Stalder et al., 2012). Among five different classes of integrons based on the sequence homology of integrases, the class 1 integrons has been reported the most in Gram-negative bacteria, including *E. coli* (Yu, Li, Liu, Zhao, & Li, 2013). In Thailand, the high prevalence of class 1 integrons among *E. coli* has been reported in hospitalized and non- hospitalized patients (Phongpaichit, Tunyapanit, & Pruekprasert, 2011), healthy human (Phongpaichit, Wuttananupan, & Samasanti, 2008), and food-producing animal (Chaisatit, Tribuddharat, Pulsrikarn, & Dejsirilert, 2012). However, little is known about genetic mechanism conferring antimicrobial resistance among MDR-DEC in view of travelers in Thailand. This study therefore aimed to determine the prevalence and characterization of class 1 integrons in MDR-DEC isolated from 2001-2004 and 2010 of international travelers in Thailand.

Materials and Methods

Bacterial strains

Forty-nine MDR-DEC isolates collected from international travelers with diarrhea (n=42) and non diarrhea (n=7) in 4 regions of Thailand (Central, West, East, and Northeast) were obtained in this study. Isolates were collected from 2001-2004 and 2010, which belong to 6 enteroaggregative *E. coli* (EAEC), 28 enteropathogenic *E. coli* (EPEC), 13 enterotoxigenic *E. coli* (ETEC), 1 enteroinvasive *E. coli* (EIEC), and 1 Shiga-like toxin producing *E. coli* (STEC). DEC isolates were kindly provided by Armed Forces Research Institute of Sciences (AFRIMS), which had been identified by using colony blot hybridization with specific digoxigenin-labeled polynucleotide probes and subjected to antimicrobial susceptibility testing (AST) by disk diffusion method that interpreting results had obtained according to the Clinical and Laboratory Standards Institute (CLSI) guidelines of each AFRIMS's year project. The antimicrobials included ampicillin (10 μg), chloramphenicol (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), streptomycin (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), sulfisoxazole (250 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and tetracycline (30 μg). MDR phenotype was considered as non-susceptible to $\geq$1 agent in $\geq$3 antimicrobial categories (Magiorakos et al., 2012).

DNA extraction

The total DNA of DEC was extracted using Gentra® Puregene® Yeast/Bact. kit (Qiagen, USA) according to manufacturer's instructions. Extracted DNA was subjected to all experiments in this study.

Integrons detection and characterization

Polymerase chain reaction (PCR)

Genomic DNA from DEC isolates were conducted in PCR with specific primers for the presence of integrase gene (*intI1*) and 5′- and 3′-conserved sequences (5′CS-3′CS) for internal segments of integron element. The intI1 primer sequences were *intI1*-F: 5′-GCCTTGCTGTTCTTCTACGG-3′ and *intI1*-R: 5′-GATGCCTGCTTGTTCTACGG-3′ (Ng, Mulvey, Martin, Peters, & Johnson, 1999), which produced a 558-bp amplicon size. PCR was performed in a 20 µl reaction mixture containing 1X standard buffer with 1.5 mM $MgCl_2$ (New England Biolabs, UK), 200 µM dNTPs, 0.5 µM each primer, 1 unit *Taq* polymerase (New England Biolabs, UK), and 1-10 ng/µl template DNA. Amplification cycles consisted of pre-denaturation at 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 68°C for 1 minute, and final extension at 68°C for 5 minutes. For 5′CS-3′CS, the primer sequences used were 5′CS: 5′-GGCATCCAAGCAGCAAG-3′ and 3′CS: 5′-AAGCAGACTTGACCTGA-3′ (Ng et al., 1999), which produced variable amplicon sizes. The reaction mixture contained 1X ThermoPol buffer with 2 mM $MgCl_2$ (New England Biolabs, UK), 1 M betain, 200 µM dNTPs, 0.5 µM each primer, 1 unit *Taq* polymerase (New England Biolabs, UK), and 1-10 ng/µl template DNA in a final volume 20 µl. The PCR was carried out with pre-denaturation at 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 68°C for 3 minutes, and final extension at 68°C for 5 minutes. *E. coli* EC114, an integron containing *dfrA17-aadA5* strain and *E. coli* Top10 were used as a positive and negative control, respectively. The PCR products were characterized for the resistance gene cassettes by cloning before sequencing using TA Cloning Kit (Invitrogen, USA). Nucleotide sequences were compared using the BLAST program supported by the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov).

Dot blot hybridization

A PCR product with specific primer to *intI1* was used to synthesize and label for *intI1* probe by using DIG High Prime DNA Labeling kit and Detection Starter I (Roche Applied Science, Germany). Hybridization procedure and condition were performed according to the manufacturer's instructions. *E. coli* EC114, an integron containing *dfrA17-aadA5* strain and *E. coli* Top10 were used as a positive and negative control, respectively.

Results

Antimicrobial resistance pattern

Of the 49 MDR-DEC isolates, all of them exhibited resistance to tetracycline and sulfisoxazole followed by ampicillin (89.8%), streptomycin (89.8%), and trimethoprim-sulfamethoxazole (87.8%), respectively. The most frequent phenotype was resistant to ampicillin-streptomycin-sulfisoxazole-tetracycline. The resistance pattern was similar in both diarrhea and non-diarrhea group. Antimicrobial resistance profile of all *E. coli* isolates is shown in Table 1.

Table 1 Antimicrobial resistance profile of DEC isolates by disk diffusion method

	Percentage of isolate with antimicrobial resistance[a] (%)												
Sample (n)	**AM**	**C**	**CTX**	**CRO**	**GN**	**K**	**N**	**S**	**NA**	**CIP**	**G**	**SXT**	**TE**
Diarrhea (42)	88.1	42.9	2.4	2.4	14.3	14.3	11.9	90.5	35.7	7.1	100	88.1	100
Non-diarrhea (7)	100	57.1	0	0	14.3	0	0	85.7	28.6	0	100	85.7	100
Total (49)	89.8	44.9	2	2	14.3	12.2	10.2	89.8	34.7	6.1	100	87.8	100

[a]AM, ampicillin; C, chloramphenicol; CTX, cefotaxime; CIP, ciprofloxacin; CRO, ceftriaxone; G, sulfisoxazole; GN, gentamicin; K, kanamycin; N, neomycin; NA, nalidixic acid; S, streptomycin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline.

Detection and characterization of class 1 integrons

PCR with specific primers for *intI1* and 5′CS-3′CS was performed for the presence of integrase gene and internal segment of integron element. Of 49 MDR-DEC isolates, 69% (29/42) and 42.9% (3/7) were *intI1* positive for isolates from diarrhea and non-diarrhea group, respectively. 5′CS-3′CS were detected in 61.9% (26/42) of traveler with diarrhea and 42.9% (3/7) of traveler without diarrhea (Table 2). PCR products showed expected size of 558 bp for *intI1* (Figure 1) and variable sizes for 5′CS-3′CS sequences (Figure 2). Dot blot hybridization was carried out to confirm the presence of class 1 integrase gene by using *intI1* specific probe. The positive results presented purple dot for each sample (Figure 3). Dot-blot hybridization could restore the PCR result for ET050 and ET052, which are non-diarrhea group (Table 3) with the detection rate of 71.4% (5/7). By using combination of all detection method, the presence of class 1 integrons was detected at rate of 75.5% (37/49). However, internal segment of integron element with specific 5′CS-3′CS primers was detected for 3 integrase negative *E. coli* isolates, whereas 8 integrase positive isolates didn't present product of 5′CS-3′CS. EA011 and EA016 isolates were selected to be characterized for resistance gene cassettes. Sequence analysis indicated the presence of gene cassettes containing *aadA2-linF*, which amplicons size was 1,947 bp (accession no. JQ414042.1) for resistant to streptomycin and lincosamide in EA011, and *aadA1* conferring resistant to streptomycin, which 1,011 bp size (accession no. FJ855126.1) were identified in EA016.

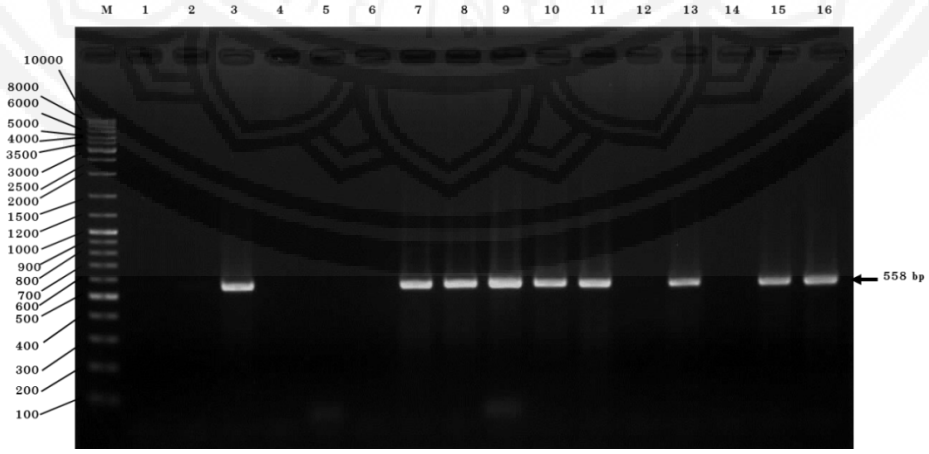

Figure 1 PCR using specific primers for *intI1*. Lane M, GeneRuler™ 1 kb DNA Ladder; Lane 1, negative control, *E. coli* Top10; Lane 2-15, samples; Lane 16, positive control, EC114 *E. coli* carrying *dfrA17* and *aadA5*.

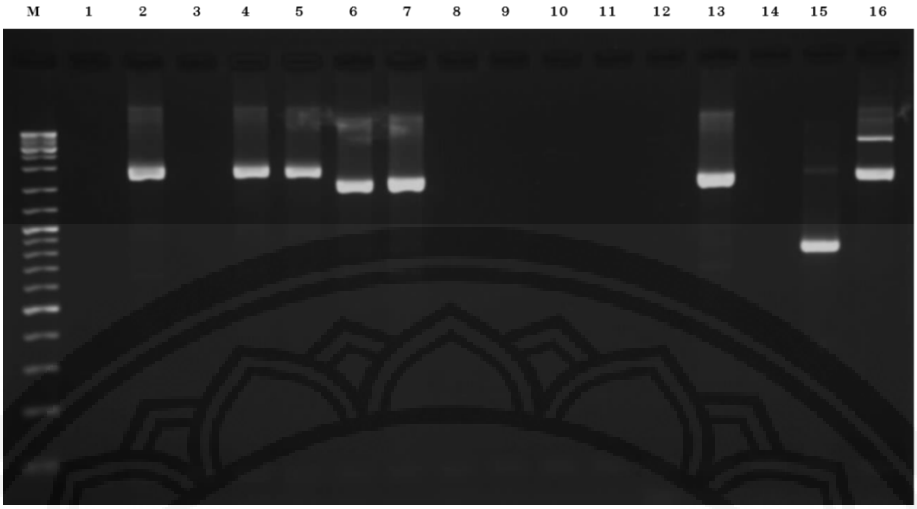

Figure 2 PCR amplification for variable size of 5′CS-3′CS. Lane M, GeneRuler™ 1 kb DNA Ladder; Lane 1, negative control, *E. coli* Top10; Lane 2-15, samples; Lane 16, positive control, EC114 *E. coli* carrying *dfrA17* and *aadA5*.

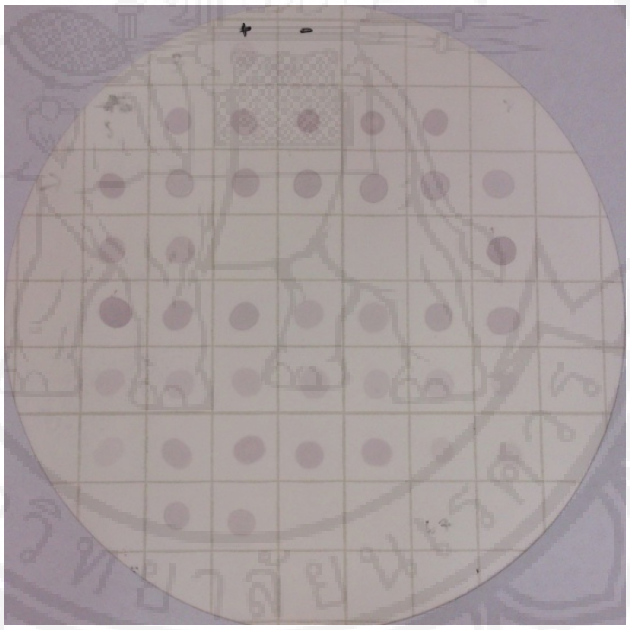

Figure 3 Dot blot hybridization in duplicates of *E. coli* isolates by using *intI1* specific probe. "+", positive control, EC114 *E. coli* carrying *dfrA17- aadA5*; and "-"; negative control, *E. coli* Top10.

Table 2 Class 1 integrons determination by PCR and dot blot hybridization

Sample (n)	Class 1 integrons by PCR		Class 1 integrons by dot blot hybridization (%)	Presence of class1 integrons (combined methods) (%)
	intI1 (%)	5′CS-3′CS (%)		
Diarrhea (42)	29 (69)	26 (61.9)	29 (69)	30 (71.4)
Non-diarrhea (7)	3 (42.9)	3 (42.9)	5 (71.4)	7 (100)
Total (49)	32 (65.3)	29 (59.2)	34 (69.4)	37 (75.5)

Table 3 Class 1 integrons determination and demographic information of MDR-DEC isolates

Strain	Year	Location[a]	Nationality[b]	Symptom[c]	Duration of stay[d]	Pathotype	Class1 integrons	Resistance phenotype[e]
EA003	2004	N	A	D	ND	EAEC	+	AM,C,S,G,SXT,TE
EA004	2004	C	A	D	ND	EAEC	+	AM,C,S,G,SXT,TE
EA005	2004	C	A	D	ND	EAEC	-	AM,C,S,G,SXT,TE
EA006	2004	C	A	D	ND	EAEC	+	AM,GN,G,SXT,TE
EA007	2004	C	A	D	ND	EAEC	+	AM,GN,G,SXT,TE
EA008	2010	C	A	D	ND	EAEC	+	AM,S,G,SXT,TE
EA009	2001	P	A	D	ND	EPEC	-	S,G,SXT,TE
EA010	2001	P	A	D	ND	EPEC	+	NA,AM,S,G,TE
EA011	2001	P	A	D	ND	EPEC	+	NA,AM,C,K,N,S,G,SXT,TE
EA012	2001	P	A	D	ND	EPEC	+	AM,C,S,G,TE
EA013	2001	P	A	D	ND	EPEC	+	C,S,G,SXT,TE
EA014	2001	P	A	D	ND	EPEC	-	AM,C,S,G,SXT,TE
EA015	2001	P	A	D	ND	EPEC	+	C,S,G,SXT,TE
EA016	2002	S	A	D	ND	EPEC	+	NA,CIP,AM,C,GN,K,S,G,SXT,
EA017	2002	S	A	D	ND	EPEC	+	NA,AM,C,K,N,S,G,SXT,TE
EA019	2002	S	A	D	ND	EPEC	+	NA,AM,S,G,SXT,TE
EA020	2002	S	A	D	ND	EPEC	+	AM,C,S,G,SXT,TE
EA021	2002	S	A	D	ND	EPEC	+	NA,AM,C,K,N,S,G,SXT,TE
EA022	2003	Pr	A	D	ND	EPEC	+	AM,C,K,N,S,G,SXT,TE
EA023	2003	Pr	A	D	ND	EPEC	+	NA,AM,C,S,G,SXT,TE
EA024	2004	N	A	D	ND	EPEC	-	AM,S,G,TE
EA026	2004	N	A	D	ND	EPEC	+	NA,AM,GN,G,SXT,TE
EA027	2001	P	A	D	ND	ETEC	+	AM,S,G,SXT,TE
EA028	2001	P	A	D	ND	ETEC	+	AM,S,G,SXT,TE
EA029	2002	S	A	D	ND	EPEC	+	NA,CIP,AM,C,S,G,SXT,TE
EA030	2002	S	A	D	ND	ETEC	-	AM,S,G,SXT,TE
EA031	2002	S	A	D	ND	ETEC	-	AM,S,G,TE
EA032	2002	S	A	D	ND	ETEC	-	AM,S,G,SXT,TE
EA033	2002	S	A	D	ND	ETEC	-	AM,S,G,SXT,TE
EA034	2002	S	A	D	ND	ETEC	-	AM,S,G,SXT,TE
EA035	2004	N	A	D	ND	ETEC	-	AM,S,G,SXT,TE
ET037	2001	B	J	D	1m	EIEC	+	AM,S,G,SXT,TE
ET042	2001	B	J	D	24d	EPEC	-	NA,S,G,SXT,TE
ET043	2001	B	E	D	3d	EPEC	-	AM,S,G,TE
ET044	2001	B	E	D	4d	EPEC	+	AM,C,K,N,S,G,SXT,TE
ET045	2001	B	J	D	3d	EPEC	+	NA,AM,C,S,G,SXT,TE,CTX,C
ET046	2002	B	J	D	3d	EPEC	+	NA,AM,GN,G,SXT,TE
ET047	2002	B	A	D	1m	EPEC	+	NA,CIP,C,GN,S,G,SXT,TE
ET048	2001	B	E	ND	2y	EPEC	+	AM,C,S,G,SXT,TE
ET049	2001	B	J	ND	1y	EPEC	+	NA,AM,C,S,G,TE
ET050	2001	B	J	ND	4y	EPEC	+	AM,C,GN,G,SXT,TE
ET051	2001	B	J	ND	7m	EPEC	+	NA,AM,S,G,SXT,TE

Table 3 (Cont.)

Strain	Year	Location[a]	Nationality[b]	Symptom[c]	Duration of stay[d]	Pathotype	Class1 integrons	Resistance phenotype[e]
ET052	2003	B	C	ND	1y	EPEC	+	AM,C,S,G,SXT,TE
ET075	2001	B	A	D	1y5m	ETEC	+	AM,S,G,SXT,TE
ET076	2002	B	J	D	3d	ETEC	+	NA,AM,S,G,SXT,TE
ET077	2002	B	J	D	7d	ETEC	+	NA,AM,S,G,SXT,TE
ET078	2002	B	J	D	8d	ETEC	+	AM,S,G,SXT,TE
ET079	2001	B	J	ND	1y	ETEC	+	AM,S,G,SXT,TE
ET089	2002	B	A	ND	3d	STEC	+	AM,S,G,SXT,TE

[a]B, Bangkok; C, Chonburi; N, Nakorn Ratchasima; P,Pitsanulok; Pr, Prachuap Kkiri Khan; S, Sakaeo province.

[b]A, American; C; Canadian; E, English; J, Japanese.

[c]D, Diarrhea; ND, Non-diarrhea.

[d]d, day; m, month; y, year; ND, no data.

[e]Abbreviations are in Table 1.

Discussion

MDR-DEC have been reported in many developing countries (Kalantar et al., 2011; Kargar et al., 2014; Meng et al., 2011; Mosquito et al., 2012; Nguyen et al., 2005), which are considered high-risk destination for travelers (Diemert, 2006). In this study, DEC isolates more than 85% were resistant to tetracycline, sulfisoxazole, ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. This similar resistance pattern was reported in commensal *E. coli* from human stool in Thailand (Phongpaichit et al., 2008). These antimicrobials are widely used in human and animals even if they are old antimicrobial compounds due to low cost and ready availability (Nguyen et al., 2005; Tadesse et al., 2012), which select the antimicrobial resistance bacteria and followed by their dissemination. We determined the presence of class 1 integrons to better understand genetic mechanism conferring antimicrobial resistance. The high prevalence of class 1 integrons in MDR-DEC was evidenced (75.5%), which was similar to that reported in Iran (Kargar et al., 2014). The higher rate of class 1 integrons was noted in dot-blot hybridization technique, when compared with PCR, suggesting higher sensitivity of the homologous probe in dot-blot hybridization. However, a drawback of this technique is that it has more complicated process and takes longer time than PCR technique. Three integrase negative isolates, which were positive for 5′CS-3′CS may indicate the possible *intI1* gene truncation. On the other hand, 8 *int11*-positive isolates which had no amplicon of 5′CS-3′CS can be explained by the long arrays containing many gene cassettes or the deletion of 3′CS caused by insertion elements (Dawes et al., 2010). Interestingly, class 1 integrons were present in all isolates from travelers without diarrhea, which all of them stayed in Thailand at least 7 months; excepting ET089, which stayed only 3 days in Thailand and returned from Cambodia (Table 3). Although only 2 representative isolates were characterized for resistance gene cassettes, *aadA* conferring resistant to streptomycin, which commonly found in class 1 integrons (Chang, Chang, & Chang, 2007) were identified in this study. It is noteworthy

that, the rare cassettes type of *aadA2-linF* conferring streptomycin and lincosamide resistance was detected in EA011 isolated from American traveler in 2001. This similar cassette previously has been reported in Norway from clinical specimen collection during 1995-1996 (Heir, Lindstedt, Leegaard, Gjernes, & Kapperud, 2004), in Malaysia, 2009-2010 (Kor, Choo, & Chew, 2013), and in Thailand from chicken meat, 2010-2011 (Chaisatit et al., 2012). This finding indicated an occurrence of resistance gene cassettes might transfers between different bacterial species in different hosts under selective pressure, which happened more than 10 years apart. To our knowledge, this is the first report of *aadA2-linF* cassette array in DEC isolated from clinical sample in Thailand. It is well known that international travel and immigration contribute to dissemination of resistant bacteria, which might be horizontal or vertical gene transfer. Further studies to investigate whether the high prevalence of class 1 integron-harboring isolates in Thailand was due to clonal spread or to horizontal gene transfer will be relevant to control of their spread.

Conclusion

This study emphasized an important role of class 1 integrons dissemination among community acquired MDR-DEC isolates in Thailand during the past 10 years and suggested the circulation of these resistance elements in this community. Therefore, further studies of the genetic and epidemiology of these organisms is essential for public health to limit the emergence of bacterial resistance.

Acknowledgements

This study was supported by grants from Siriraj graduate thesis scholarship from Faculty of Medicine Siriraj Hospital, Mahidol University and Armed Forces Health Surveillance Center-Global Emerging Infections Surveillance (AFHSC-GEIS), Washington, DC, USA.

Disclaimers

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. Government.

References

Cambray, G., Guerout, A. M., & Mazel, D. (2010). Integrons. *Annu Rev Genet, 44*, 141-166. doi: 10.1146/annurev-genet-102209-163504

Chaisatit, C., Tribuddharat, C., Pulsrikarn, C., & Dejsirilert, S. (2012). Molecular characterization of antibiotic-resistant bacteria in contaminated chicken meat sold at supermarkets in Bangkok, Thailand. *Jpn J Infect Dis, 65*(6), 527-534.

Chang, L. L., Chang, T. M., & Chang, C. Y. (2007). Variable gene cassette patterns of class 1 integron-associated drug-resistant *Escherichia coli* in Taiwan. *Kaohsiung J Med Sci, 23*(6), 273-280. doi: 10.1016/S1607-551X(09)70409-7

Dawes, F. E., Kuzevski, A., Bettelheim, K. A., Hornitzky, M. A., Djordjevic, S. P., & Walker, M. J. (2010). Distribution of class 1 integrons with IS26-mediated deletions in their 3′-conserved segments in *Escherichia coli* of human and animal origin. *PLoS One, 5*(9), e12754. doi: 10.1371/journal.pone.0012754

de la Cabada Bauche, J., & Dupont, H. L. (2011). New Developments in Traveler's Diarrhea. *Gastroenterol Hepatol (N Y), 7*(2), 88-95.

Diemert, D. J. (2006). Prevention and self-treatment of traveler's diarrhea. *Clin Microbiol Rev, 19*(3), 583-594. doi: 10.1128/CMR.00052-05

Farthing, M., Salam, M. A., Lindberg, G., Dite, P., Khalif, I., Salazar-Lindo, E., . . . Wgo. (2013). Acute diarrhea in adults and children: a global perspective. *J Clin Gastroenterol, 47*(1), 12-20. doi: 10.1097/MCG.0b013e31826df662

Guiral, E., Mendez-Arancibia, E., Soto, S. M., Salvador, P., Fabrega, A., Gascon, J., & Vila, J. (2011). CTX-M-15-producing enteroaggregative *Escherichia coli* as cause of travelers' diarrhea. *Emerg Infect Dis, 17*(10), 1950-1953. doi: 10.3201/eid1710.110022

Heir, E., Lindstedt, B. A., Leegaard, T. M., Gjernes, E., & Kapperud, G. (2004). Prevalence and characterization of integrons in blood culture Enterobacteriaceae and gastrointestinal *Escherichia coli* in Norway and reporting of a novel class 1 integron-located lincosamide resistance gene. *Ann Clin Microbiol Antimicrob, 3*, 12. doi: 10.1186/1476-0711-3-12

Kalantar, E., Soheili, F., Salimi, H., & Soltan Dallal, M. M. (2011). Frequency, antimicrobial susceptibility and plasmid profiles of *Escherichia coli* pathotypes obtained from children with acute diarrhea. *Jundishapur J Microbiol, 4*(1), 23-28.

Kargar, M., Mohammadalipour, Z., Doosti, A., Lorzadeh, S., & Japoni-Nejad, A. (2014). High Prevalence of Class 1 to 3 Integrons Among Multidrug-Resistant Diarrheagenic *Escherichia coli* in Southwest of Iran. *Osong Public Health Res Perspect, 5*(4), 193-198. doi: 10.1016/j.phrp.2014.06.003

Kor, S. B., Choo, Q. C., & Chew, C. H. (2013). New integron gene arrays from multiresistant clinical isolates of members of the Enterobacteriaceae and *Pseudomonas aeruginosa* from hospitals in Malaysia. *J Med Microbiol, 62*(Pt 3), 412-420. doi: 10.1099/jmm.0.053645-0

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., . . . Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect, 18*(3), 268-281. doi: 10.1111/j.1469-0691.2011.03570.x

Meng, C. Y., Smith, B. L., Bodhidatta, L., Richard, S. A., Vansith, K., Thy, B., . . . Mason, C. J. (2011). Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. *Pediatr Infect Dis J, 30*(4), 331-335. doi: 10.1097/INF.0b013e3181fb6f82

Mosquito, S., Ruiz, J., Pons, M. J., Durand, D., Barletta, F., & Ochoa, T. J. (2012). Molecular mechanisms of antibiotic resistance in diarrhoeagenic *Escherichia coli* isolated from children. *Int J Antimicrob Agents, 40*(6), 544-548. doi: 10.1016/j.ijantimicag.2012.07.021

Ng, L. K., Mulvey, M. R., Martin, I., Peters, G. A., & Johnson, W. (1999). Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104.

Antimicrob Agents Chemother, *43*(12), 3018-3021.

Nguyen, T. V., Le, P. V., Le, C. H., & Weintraub, A. (2005). Antibiotic resistance in diarrheagenic *Escherichia coli* and *Shigella* strains isolated from children in Hanoi, Vietnam. *Antimicrob Agents Chemother*, *49*(2), 816-819. doi: 10.1128/AAC.49.2.816-819.2005

Phongpaichit, S., Tunyapanit, W., & Pruekprasert, P. (2011). Antimicrobial resistance, class 1 integrons and extended-spectrum B-lactamases in *Escherichia coli* clinical isolates from patients in South Thailand *Journal of Health Science*, *57*(3), 281-288.

Phongpaichit, S., Wuttananupan, K., & Samasanti, W. (2008). Class 1 integrons and multidrug resistance among *Escherichia coli* isolates from human stools. *Southeast Asian J Trop Med Public Health*, *39*(2), 279-287.

Stalder, T., Barraud, O., Casellas, M., Dagot, C., & Ploy, M. C. (2012). Integron involvement in environmental spread of antibiotic resistance. *Front Microbiol*, *3*, 119. doi: 0.3389/fmicb. 2012.00119

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerg Infect Dis*, *18*(5), 741-749. doi: 10.3201/eid1805.111153

Yu, G., Li, Y., Liu, X., Zhao, X., & Li, Y. (2013). Role of integrons in antimicrobial resistance: A review. *African Journal of Microbiology Research*, *7*(15), 1301-1310. doi: 10.5897/AJMR11.1568